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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

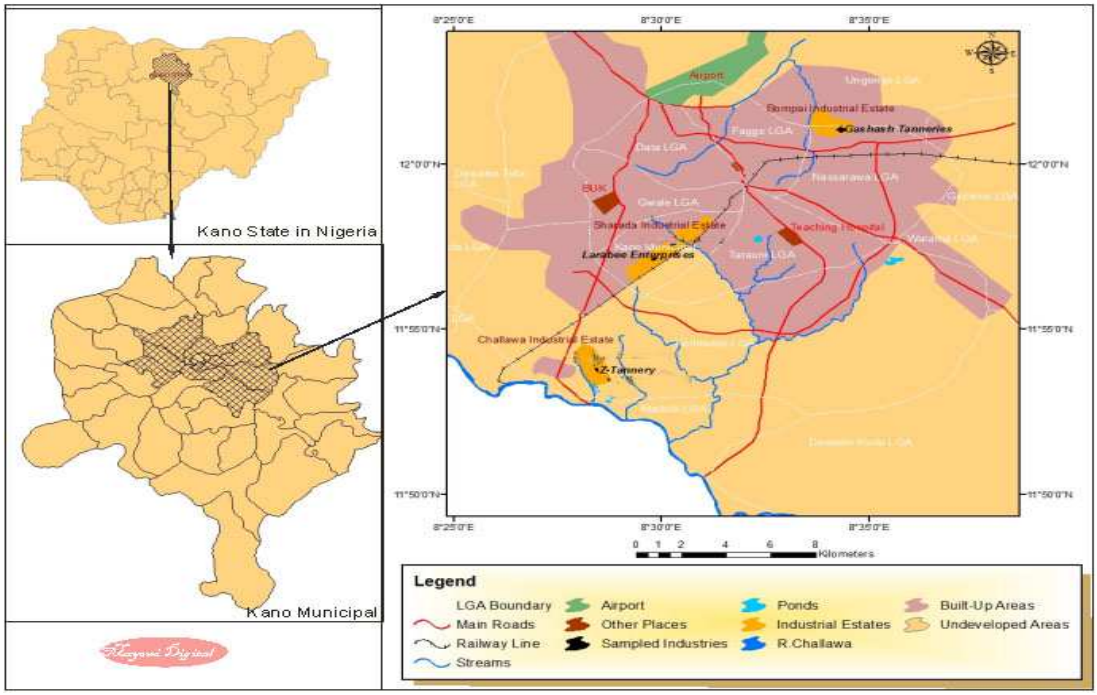

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

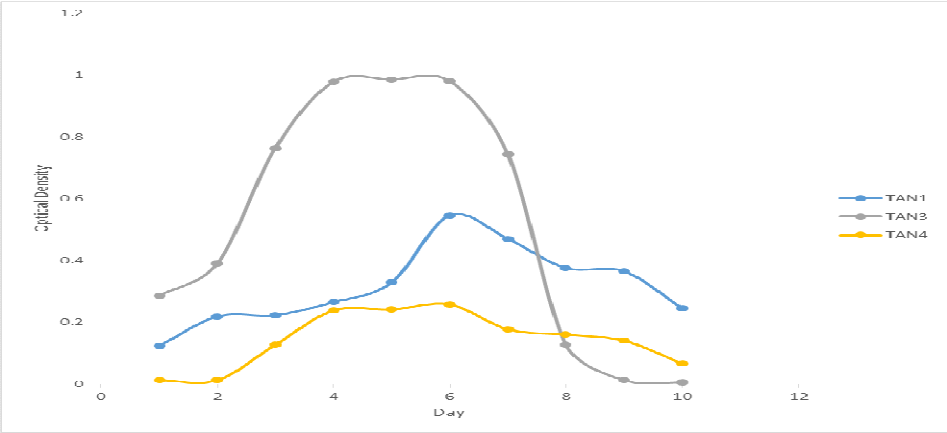

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

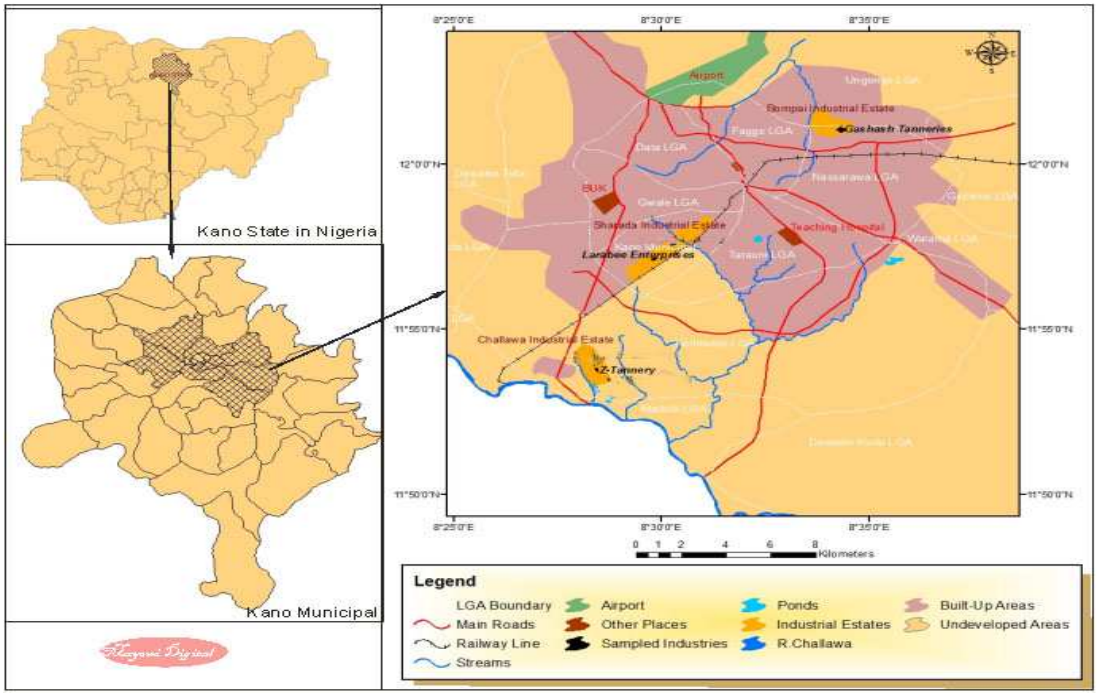

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

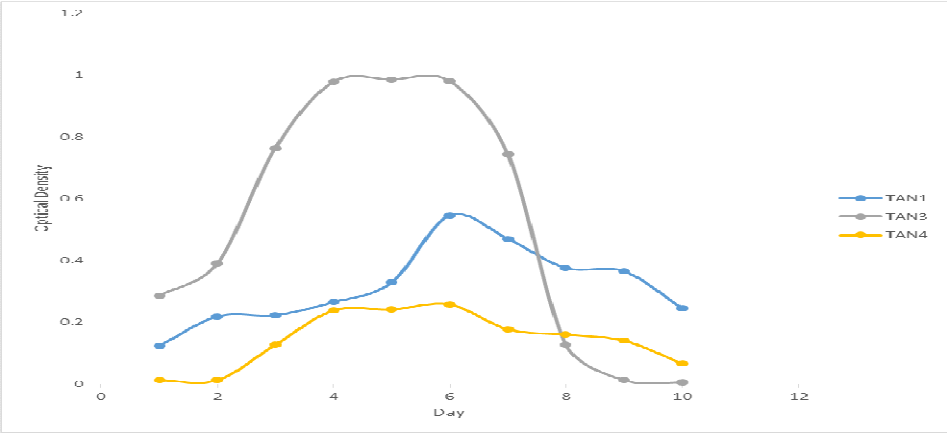

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

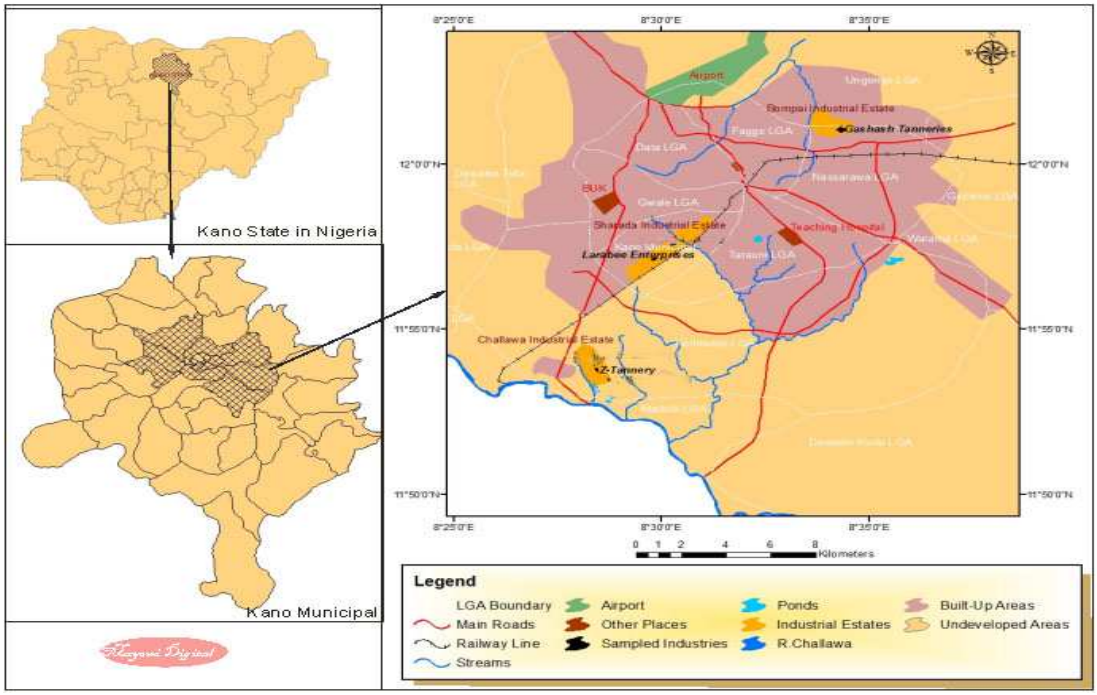

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

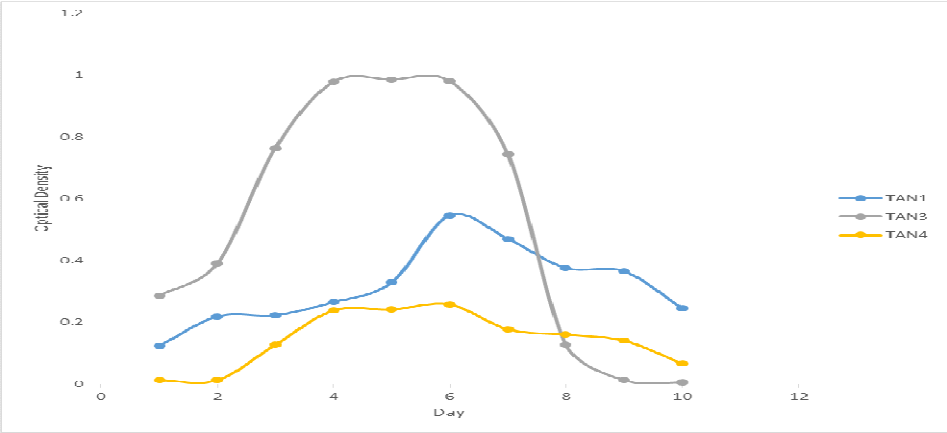

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

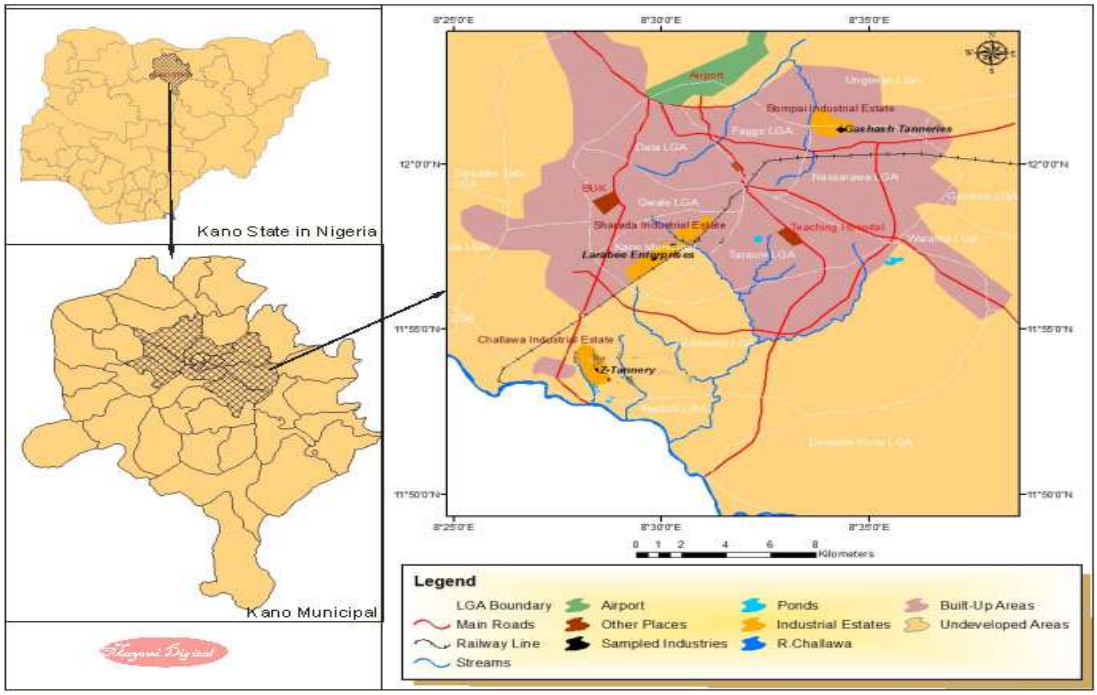

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

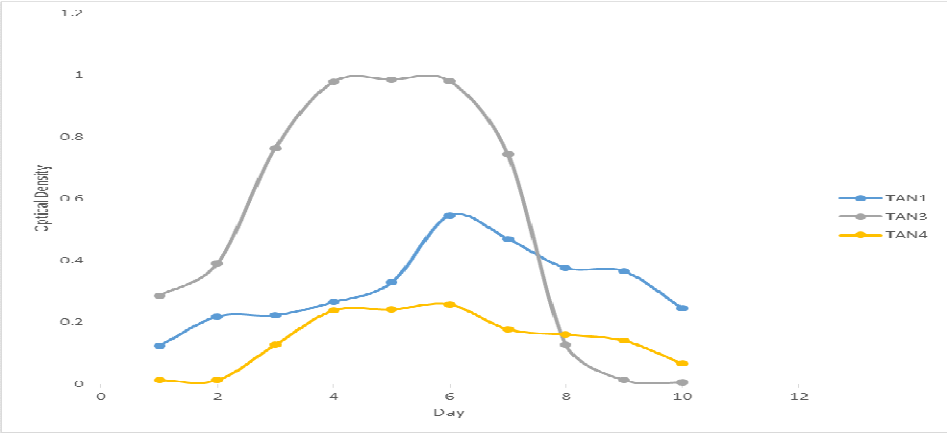

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

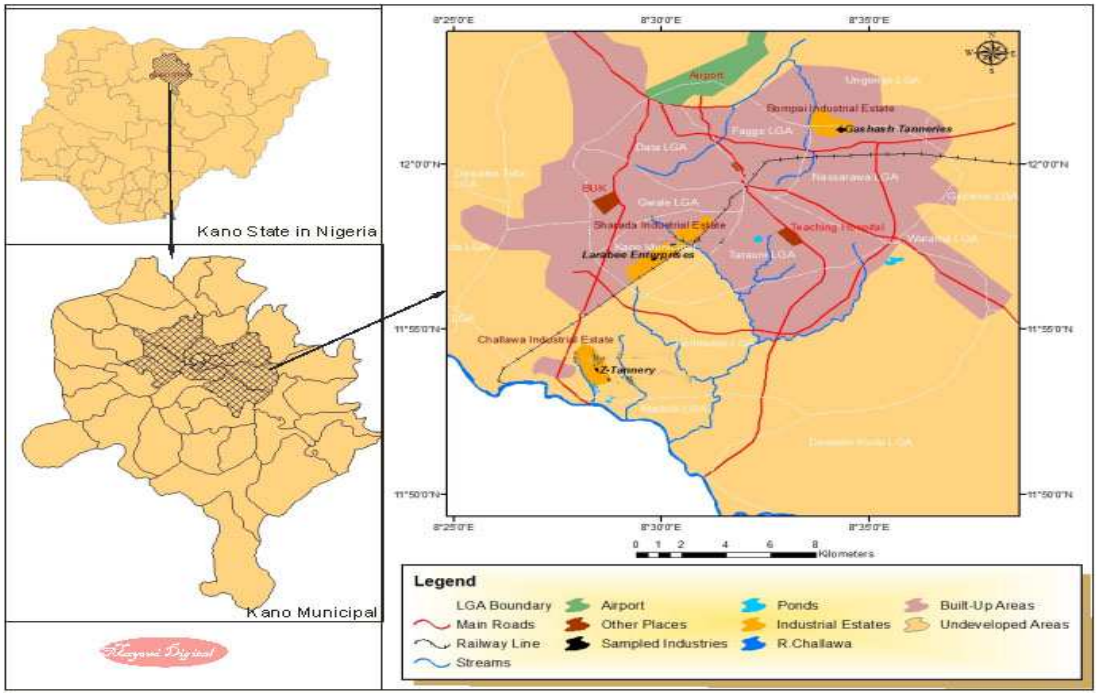

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

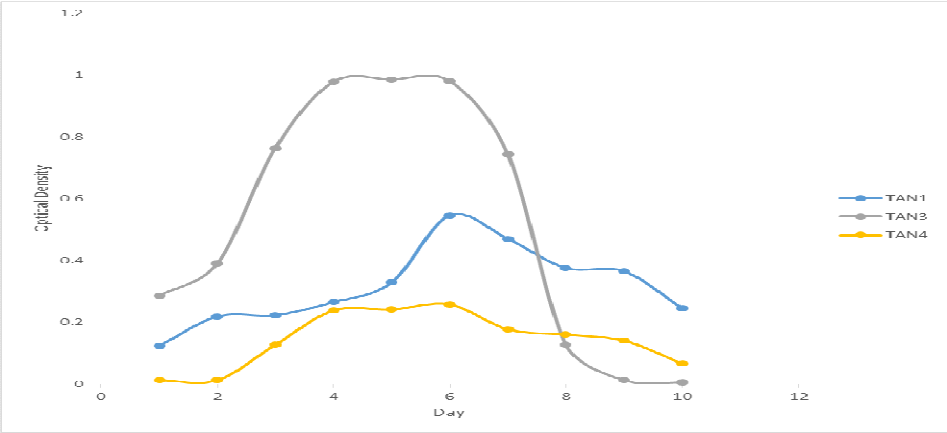

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

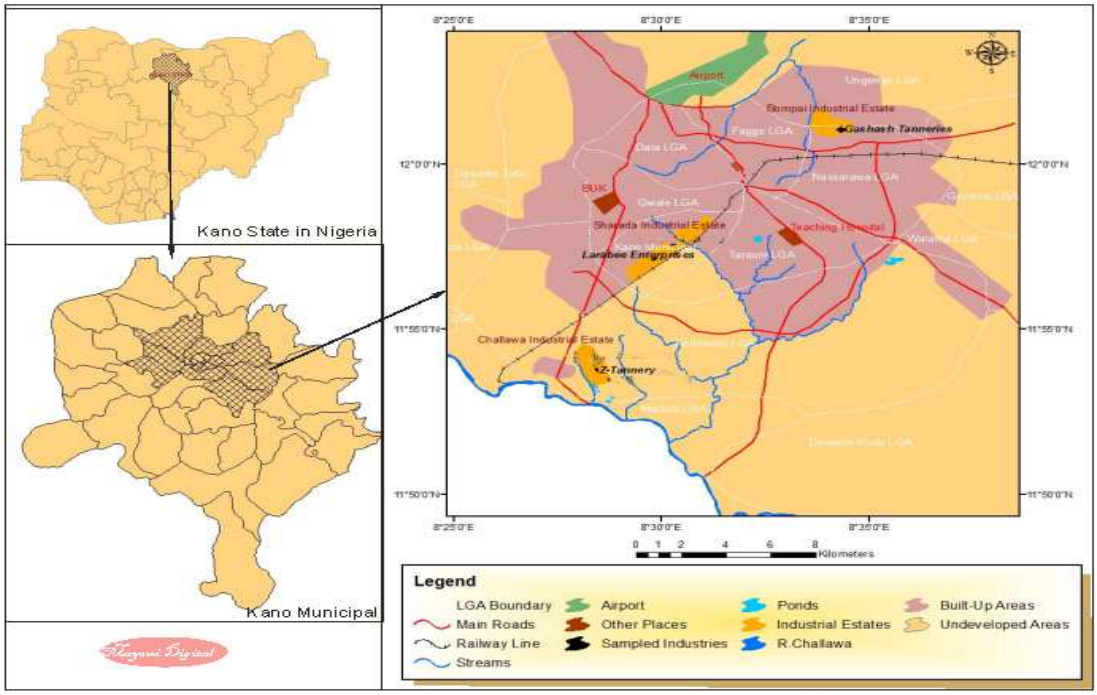

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

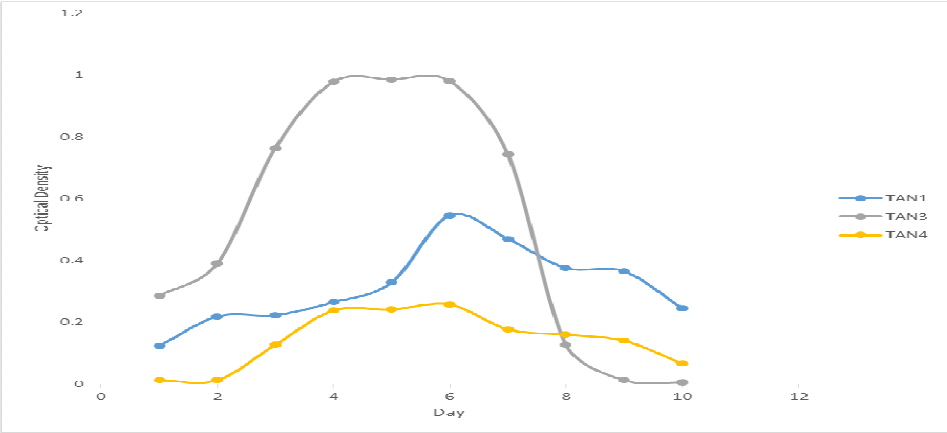

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

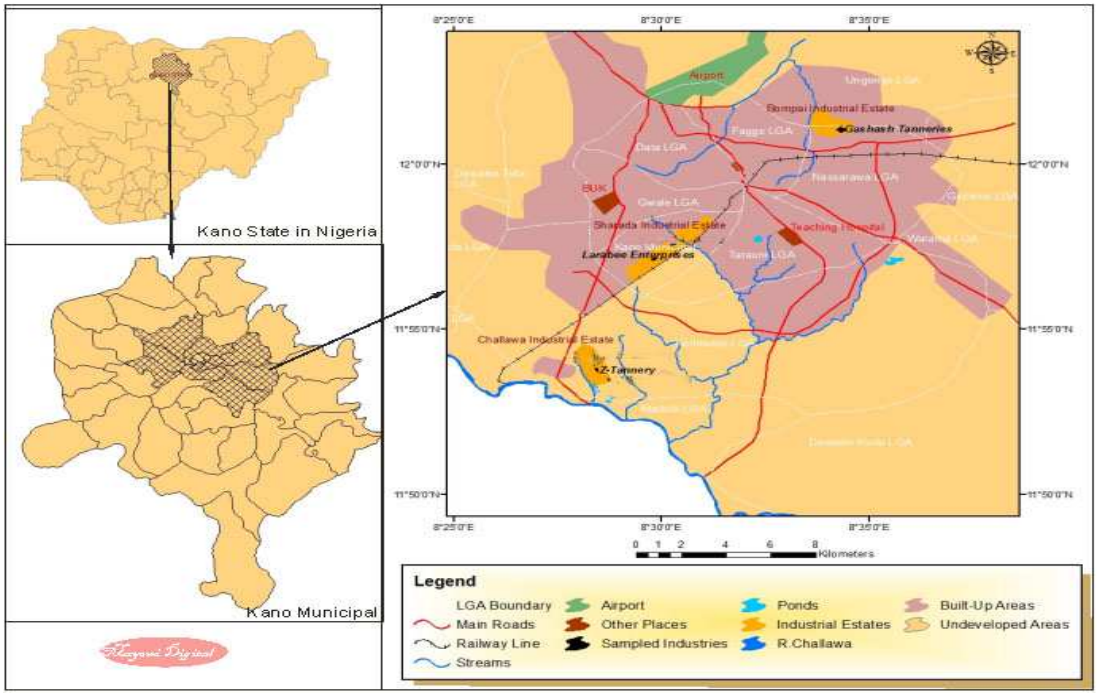

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

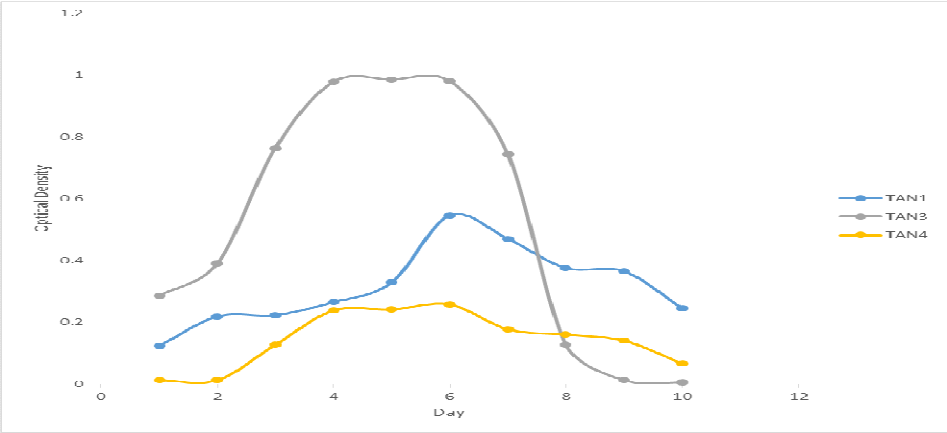

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

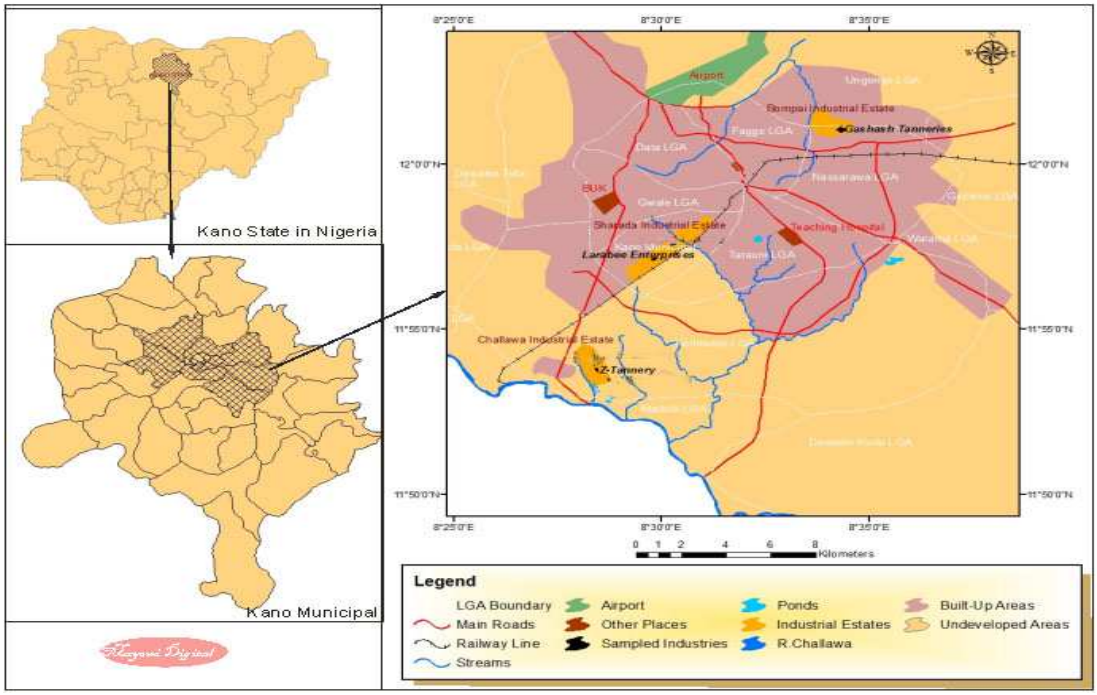

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

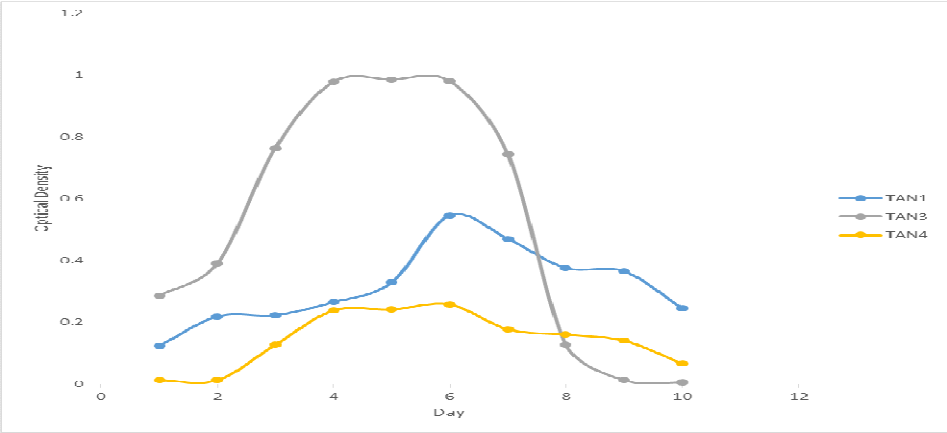

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

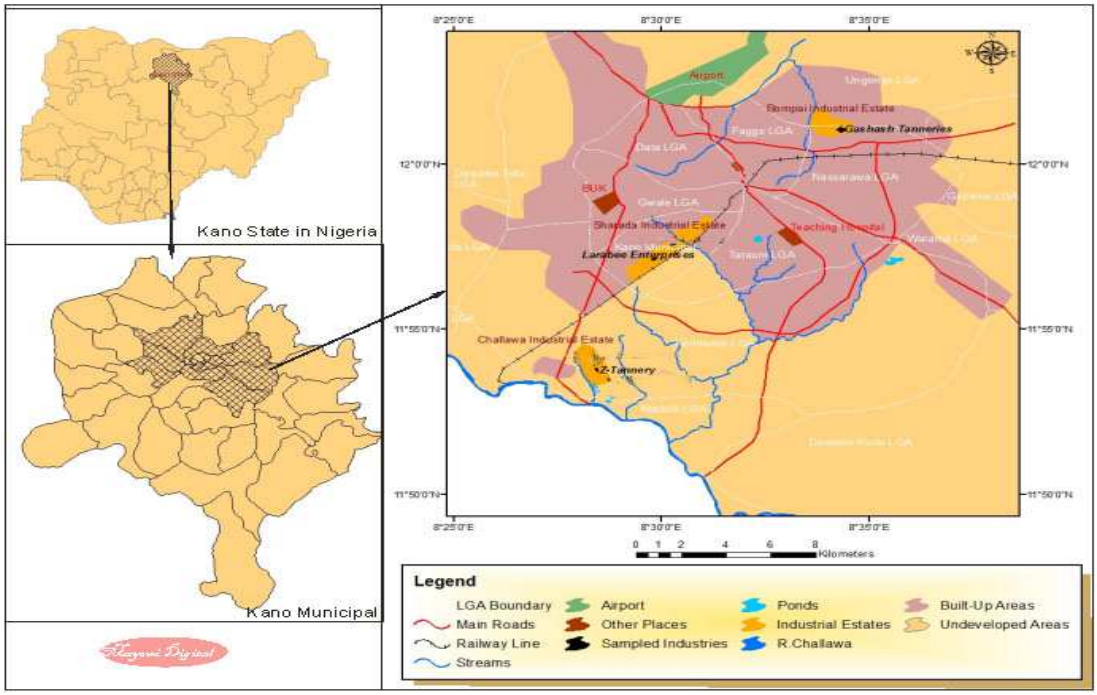

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

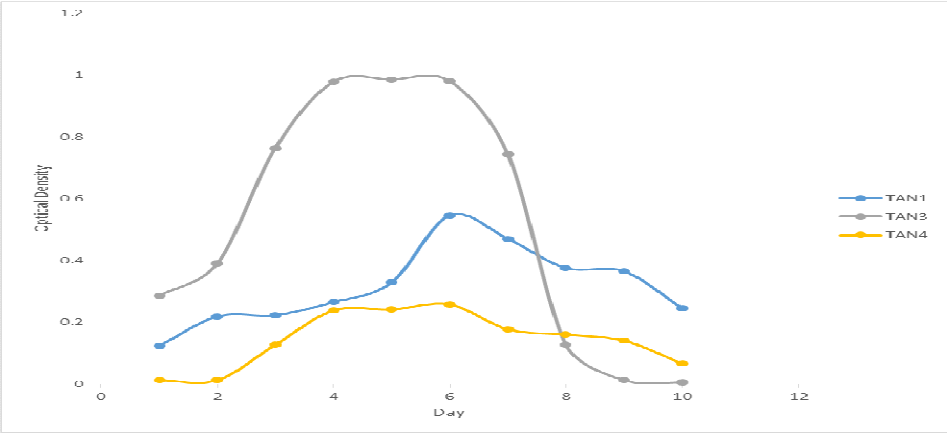

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

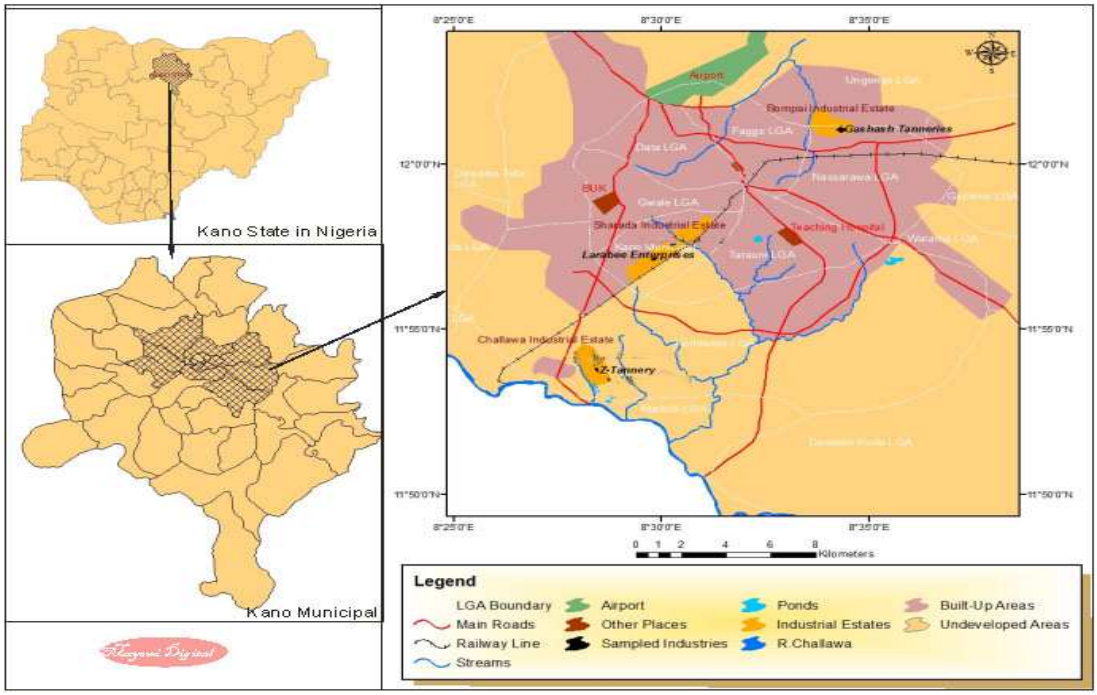

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

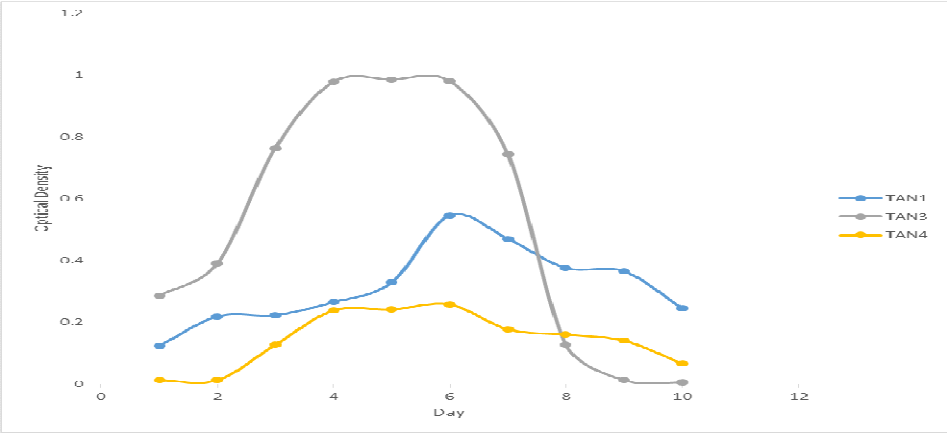

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

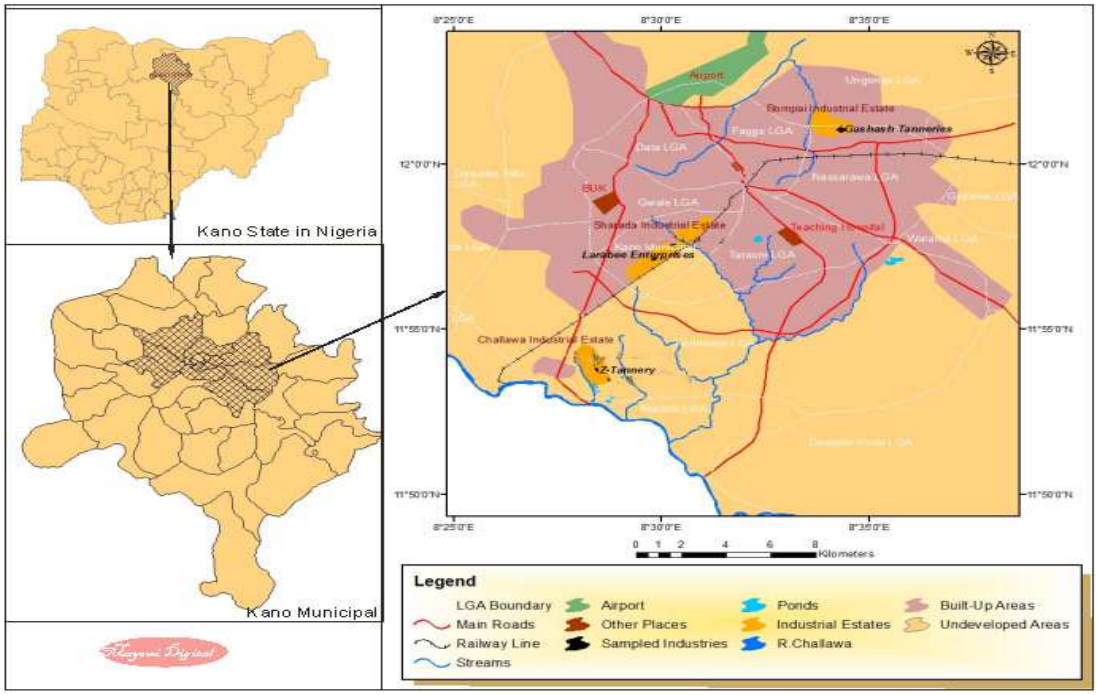

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

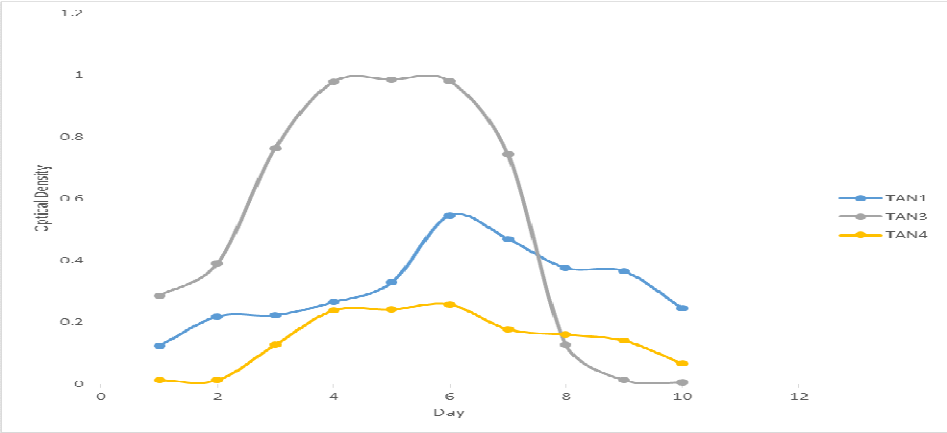

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

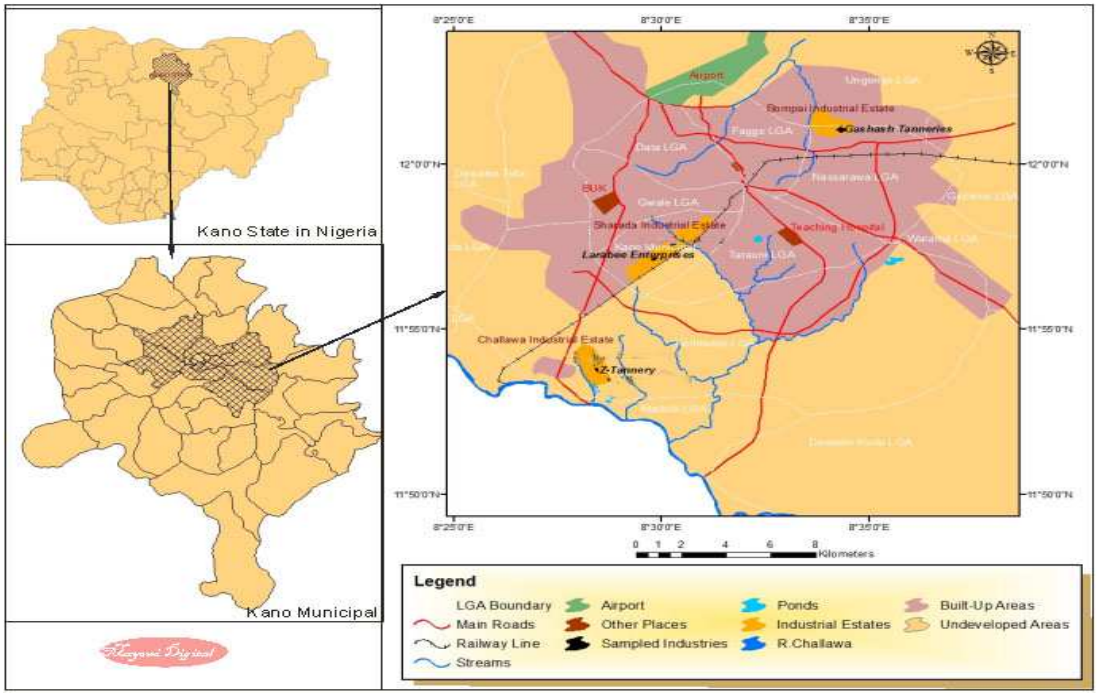

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

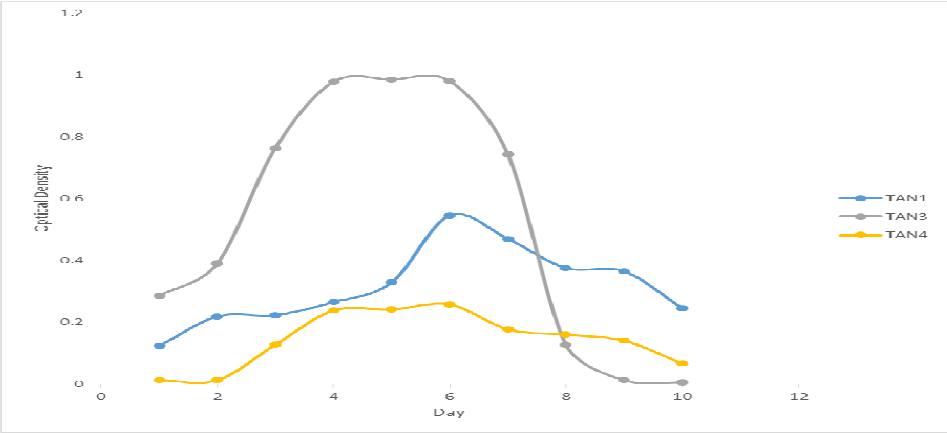

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

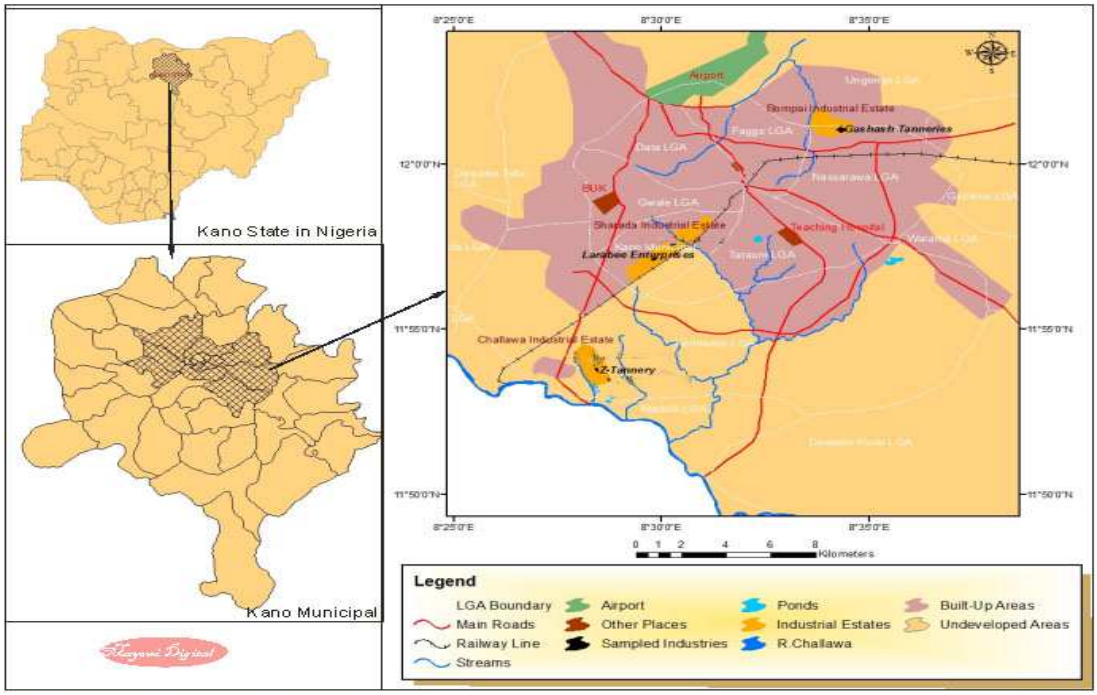

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

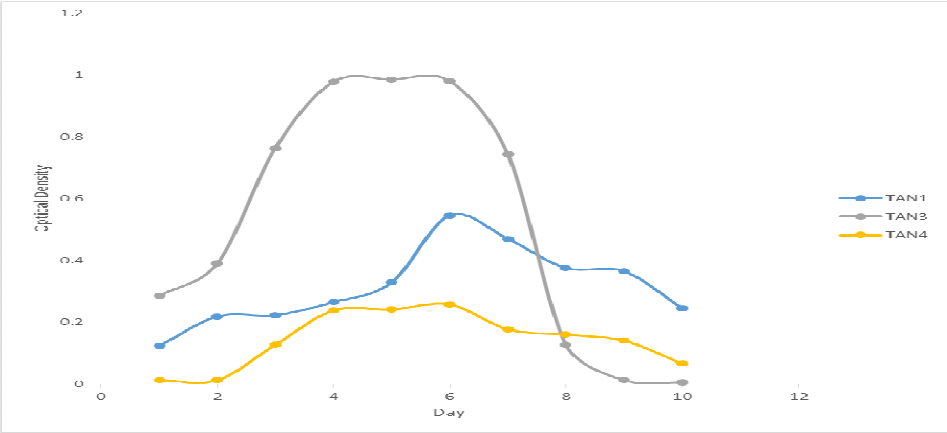

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

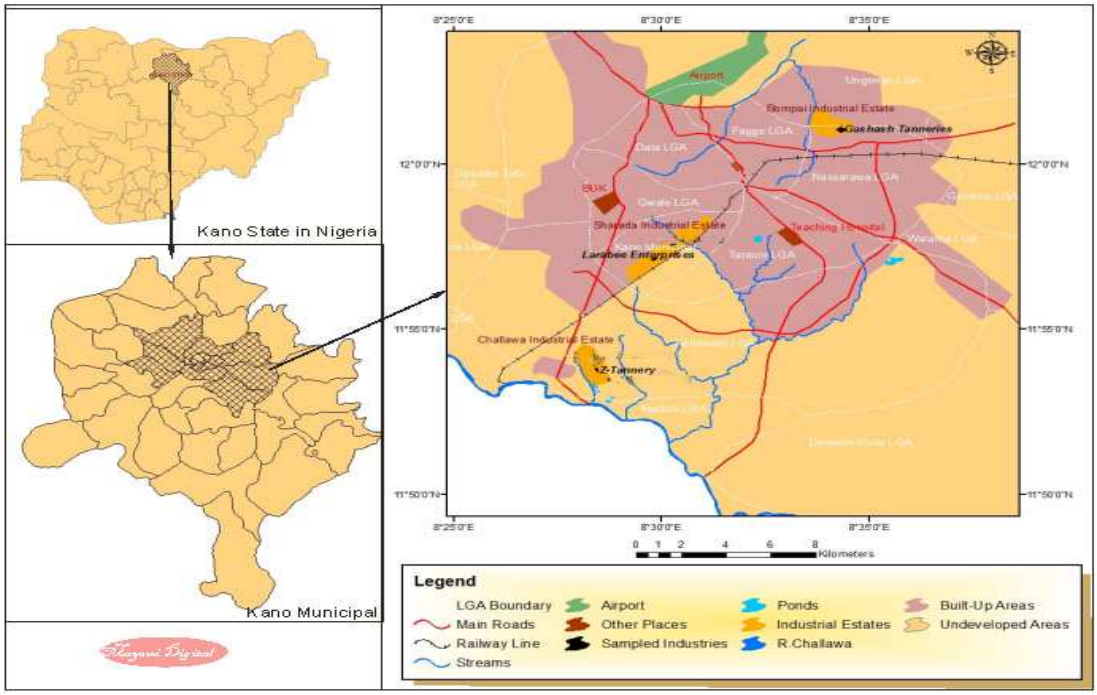

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

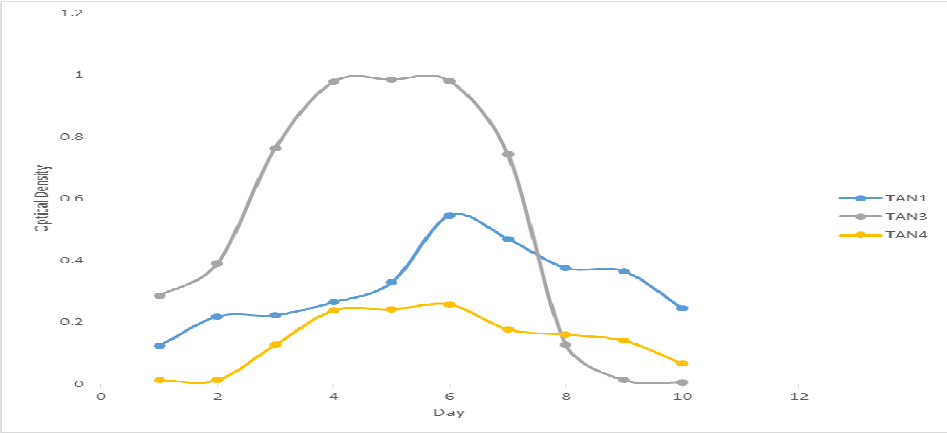

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

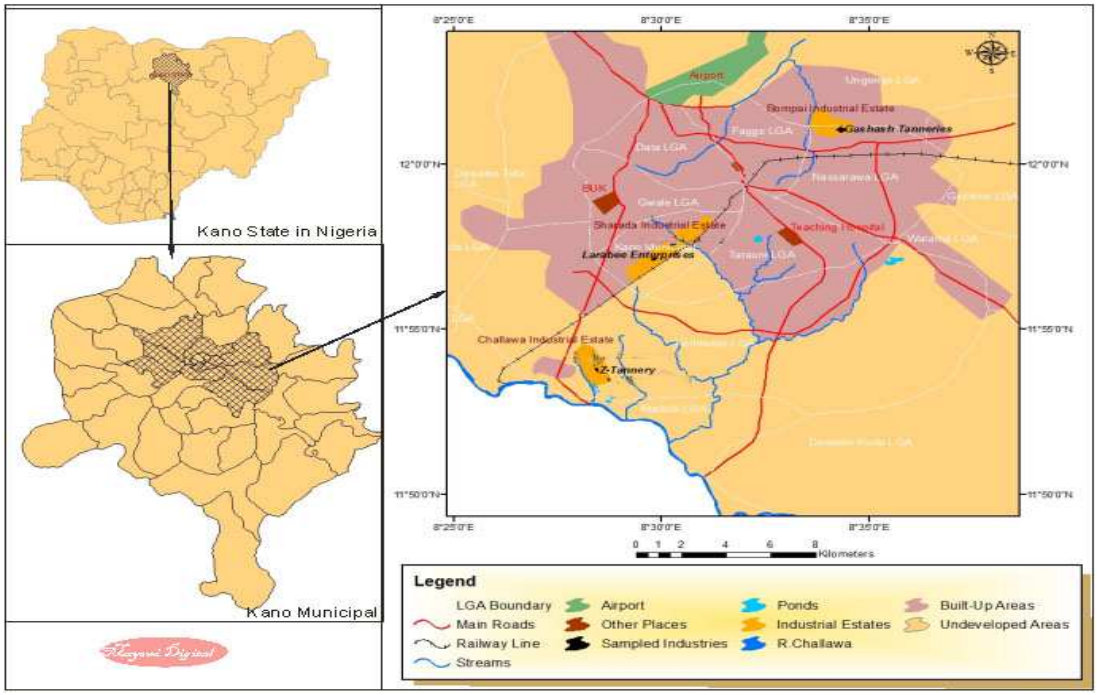

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

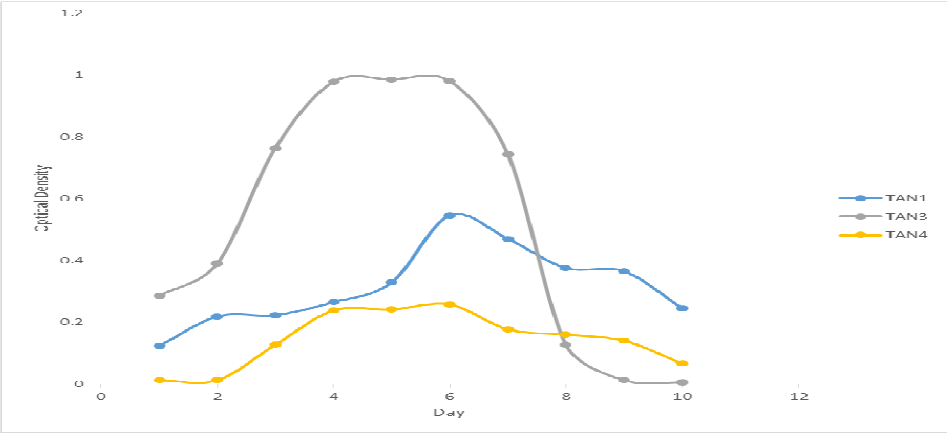

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

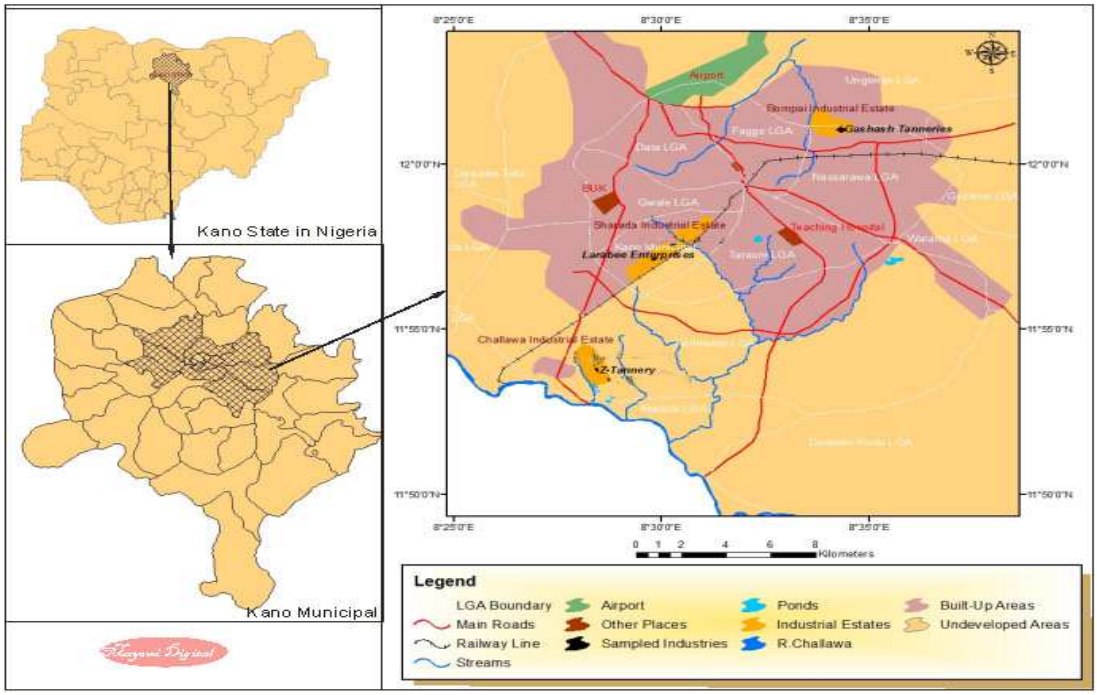

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

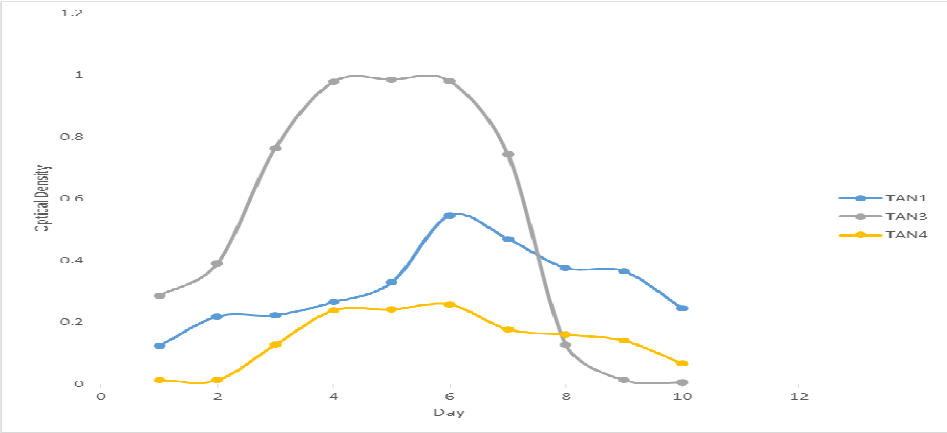

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

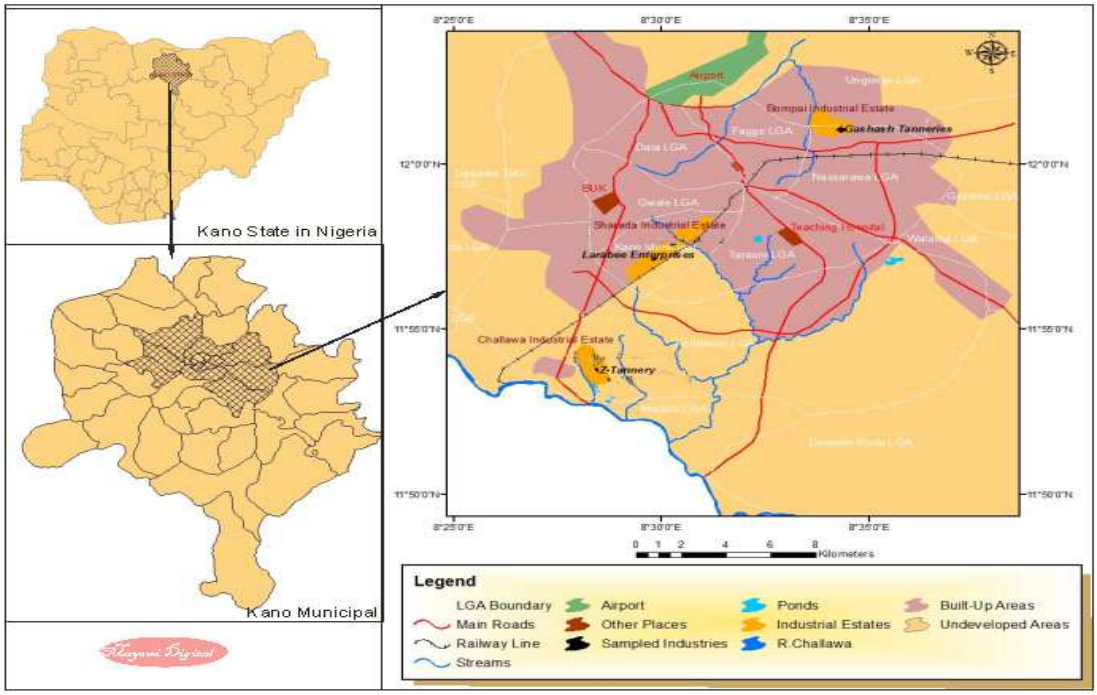

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

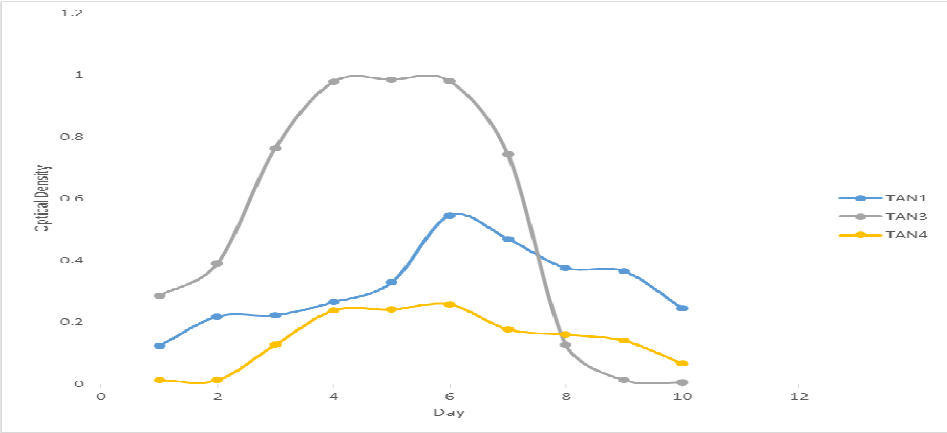

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

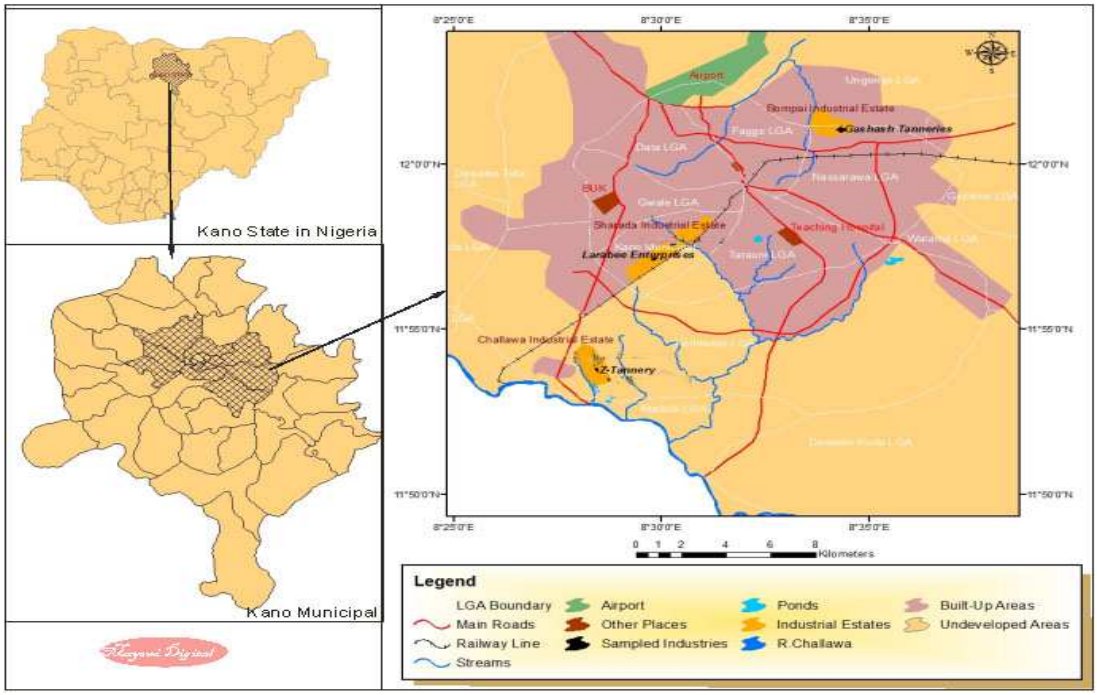

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

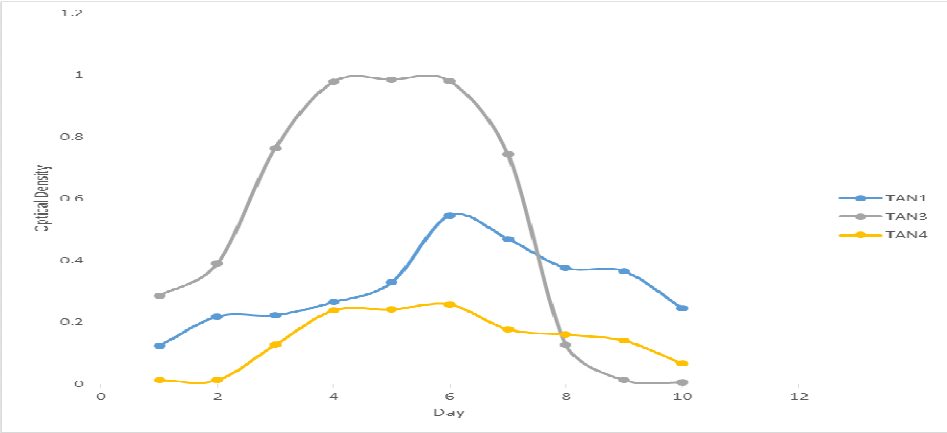

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

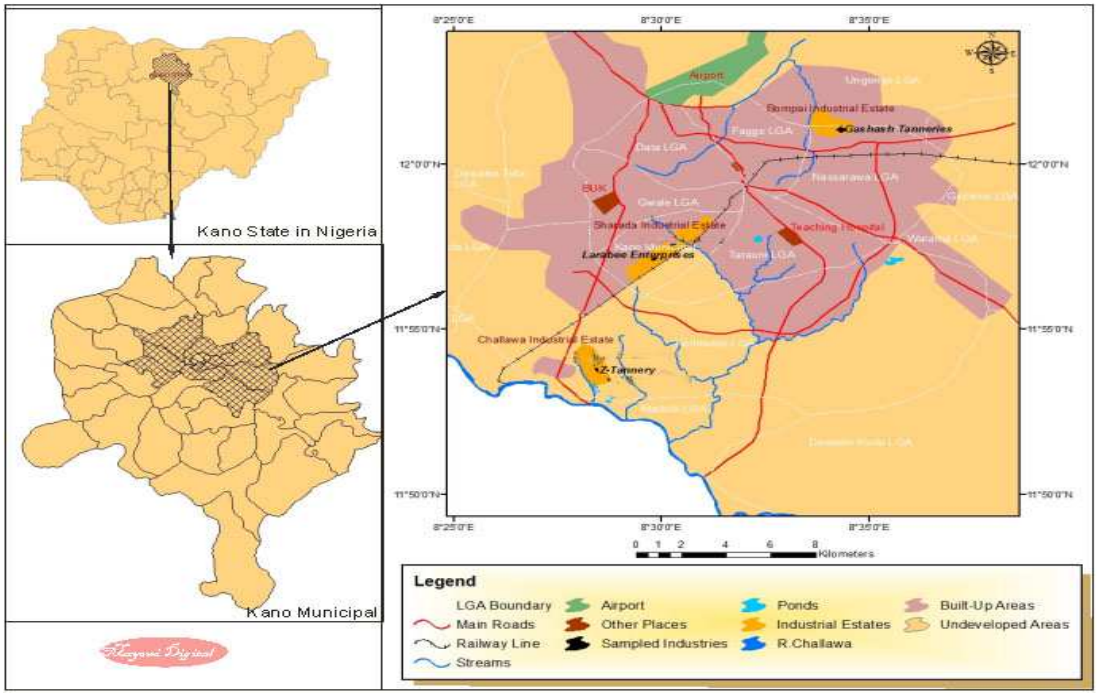

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

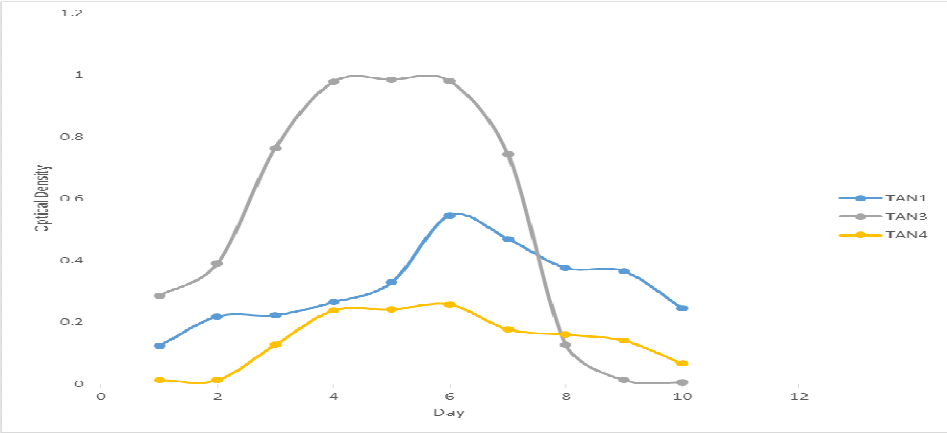

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

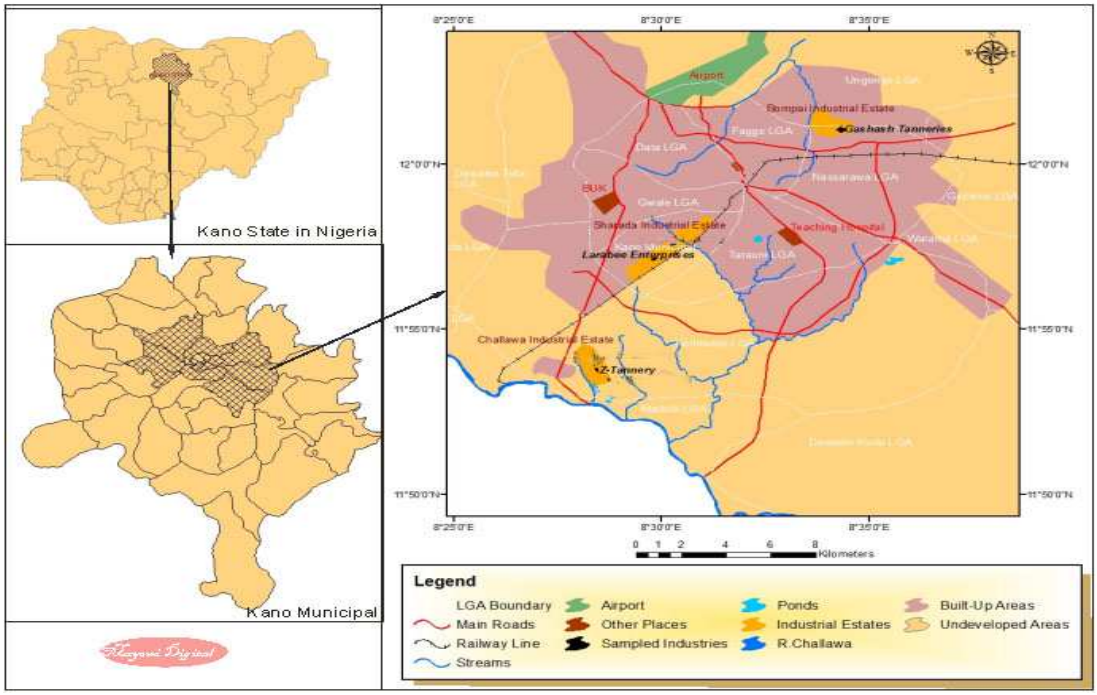

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

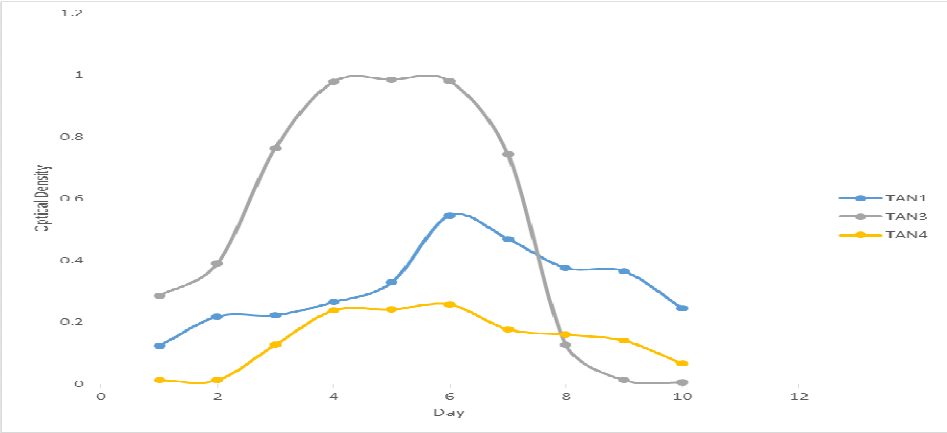

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

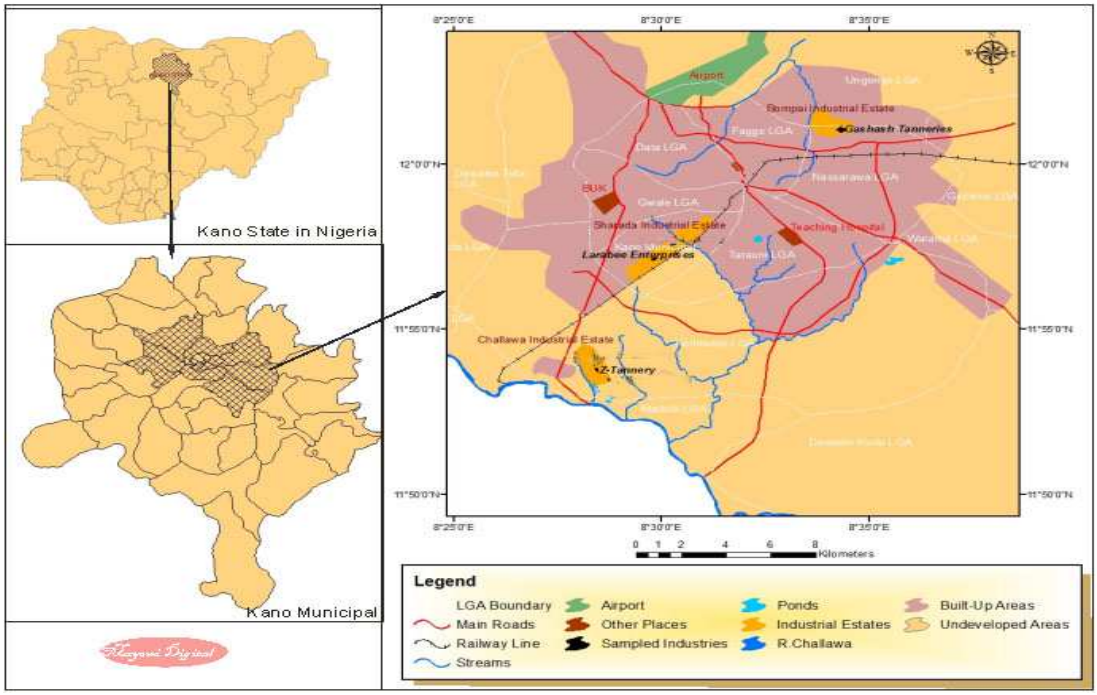

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

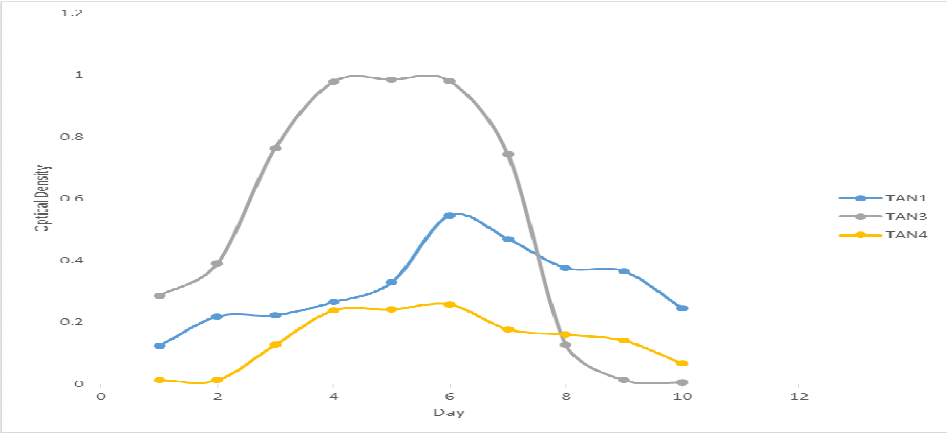

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

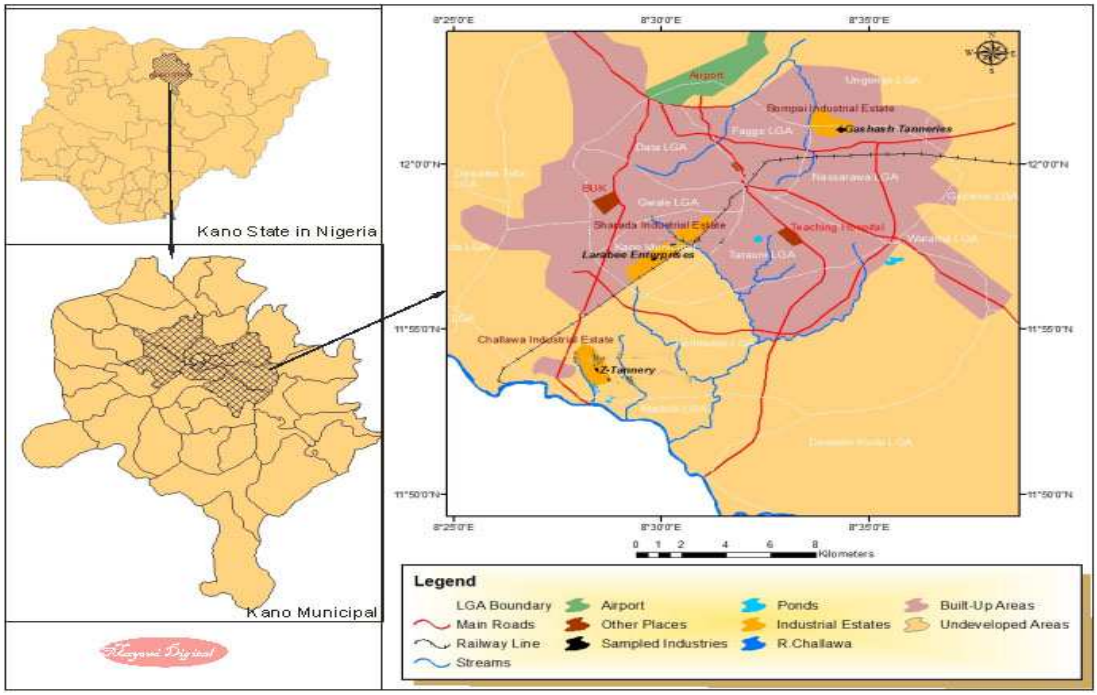

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

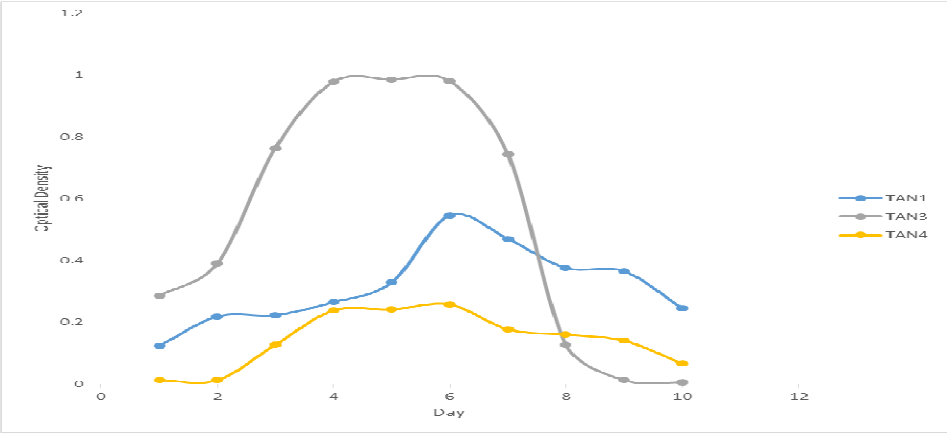

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

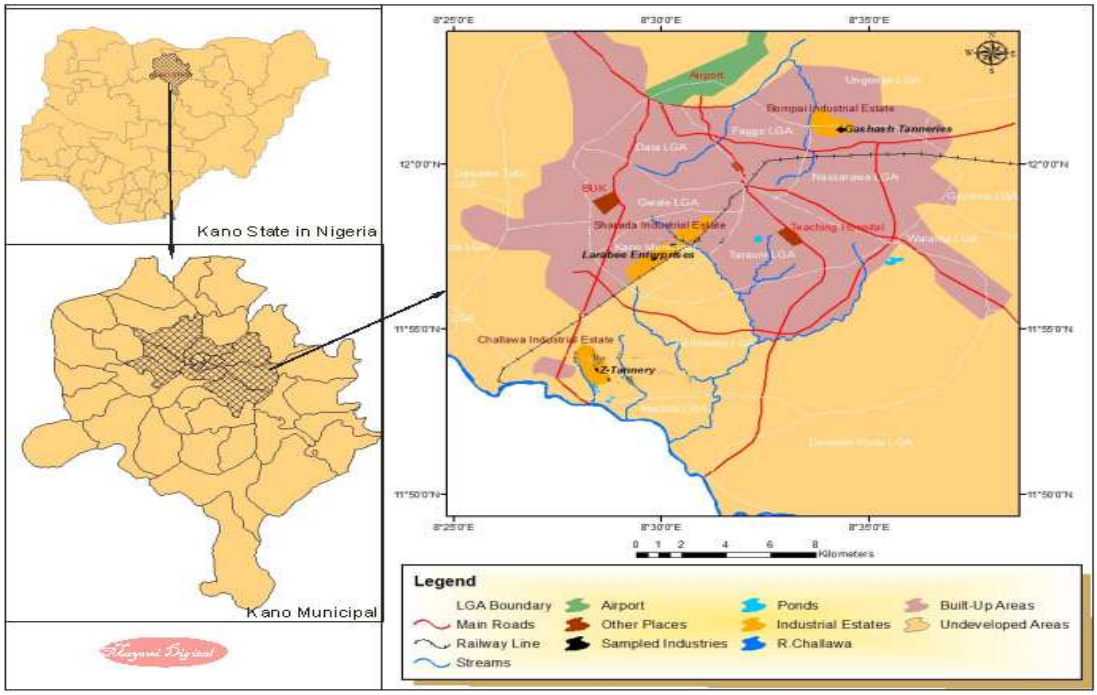

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

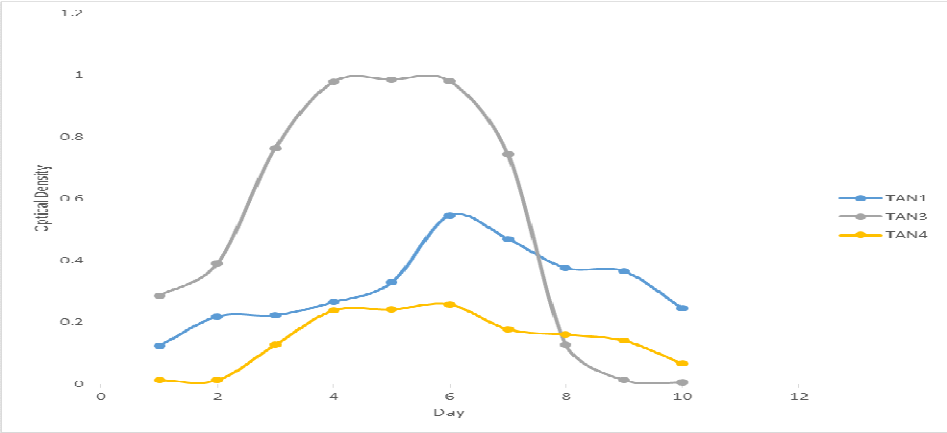

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

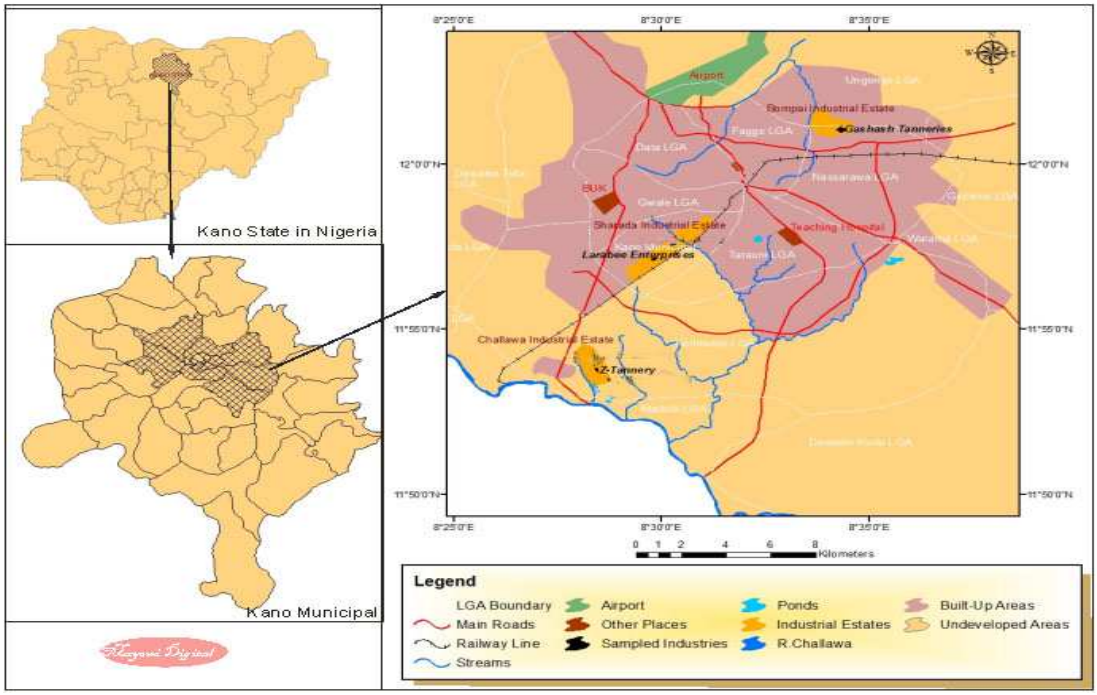

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

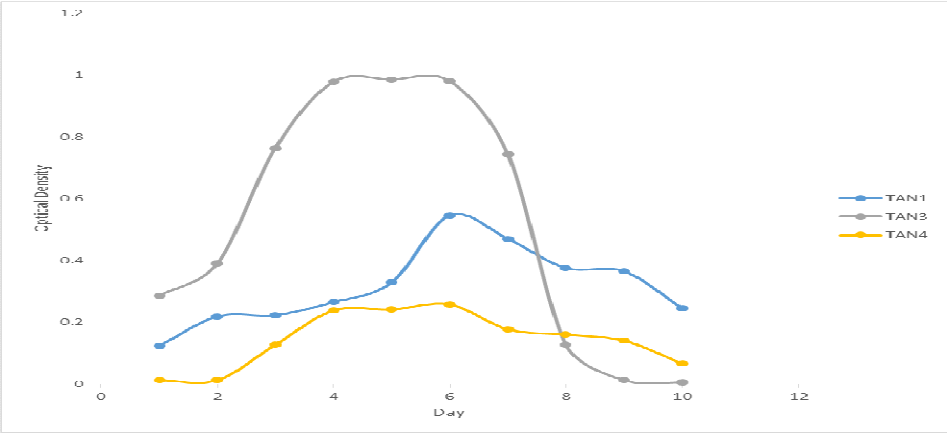

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

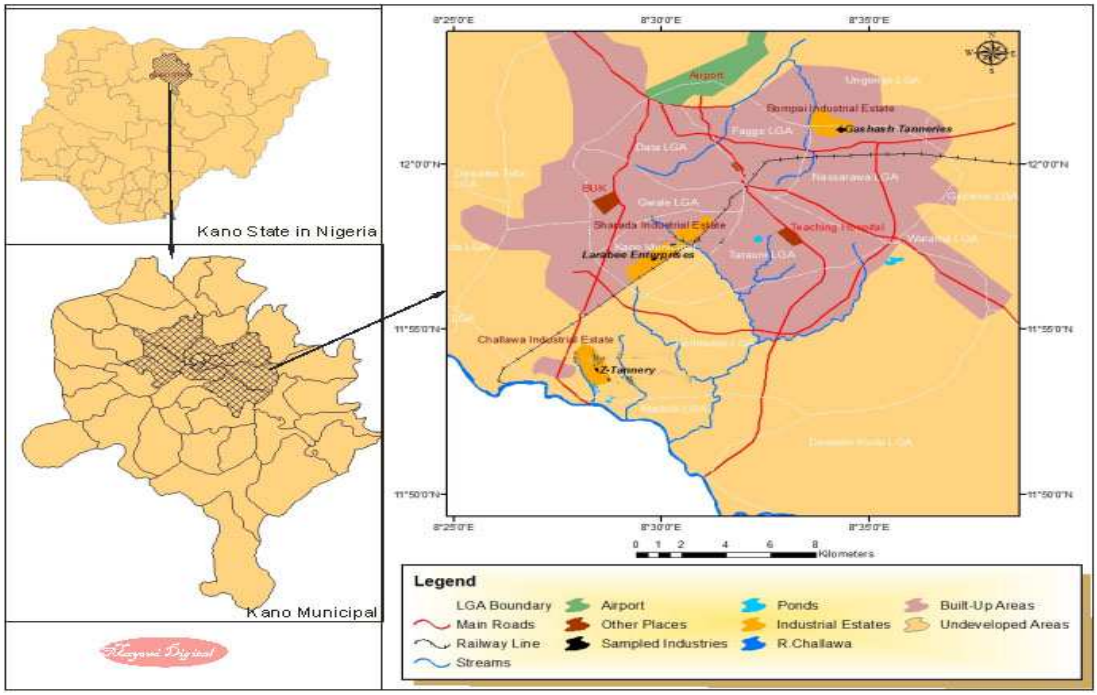

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

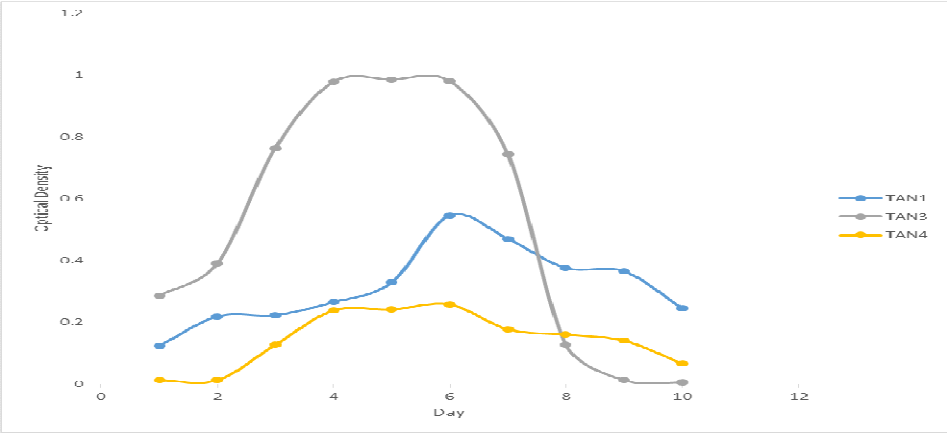

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

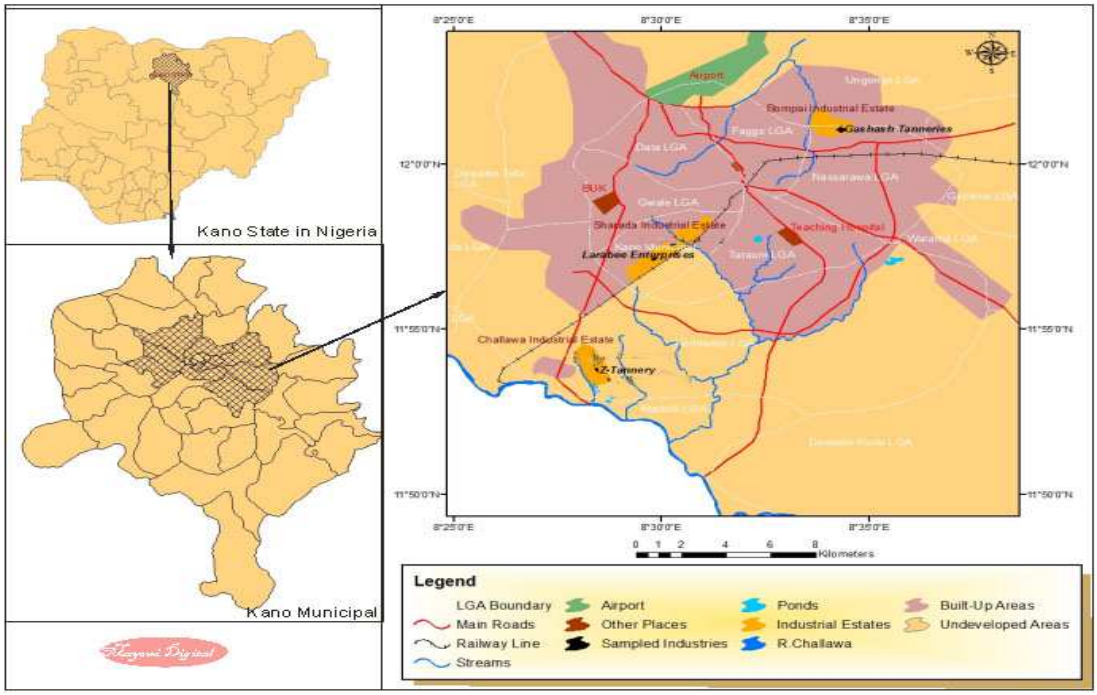

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

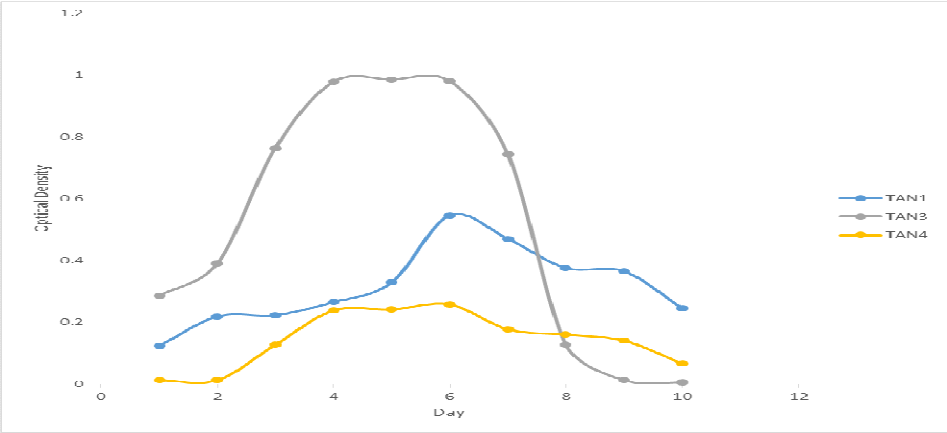

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

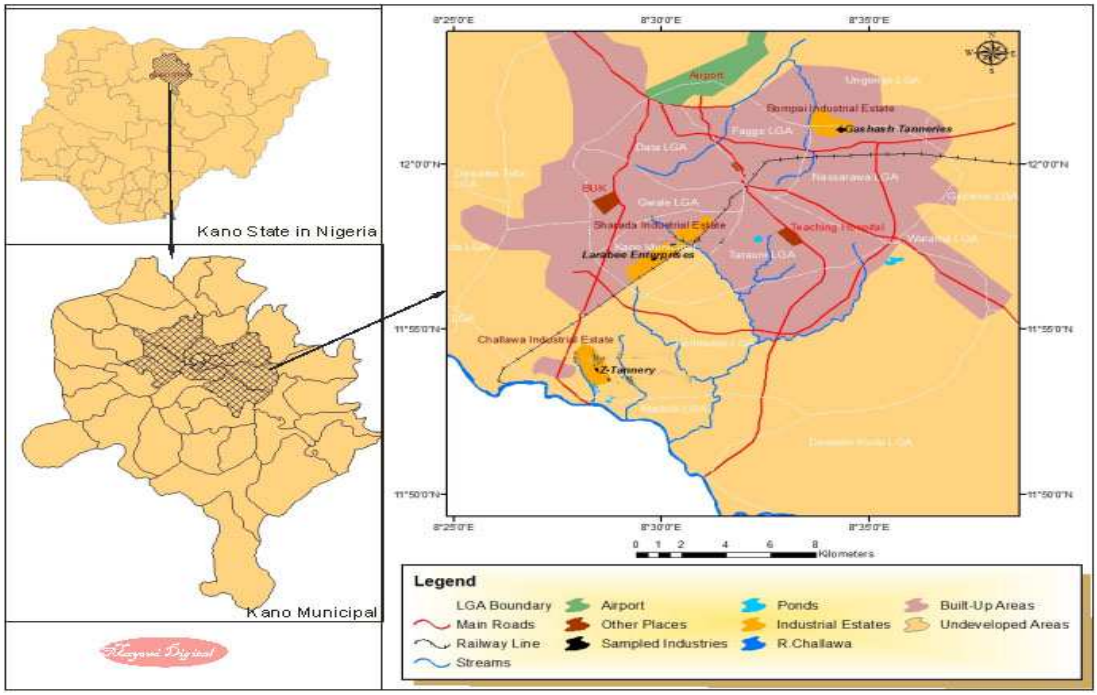

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

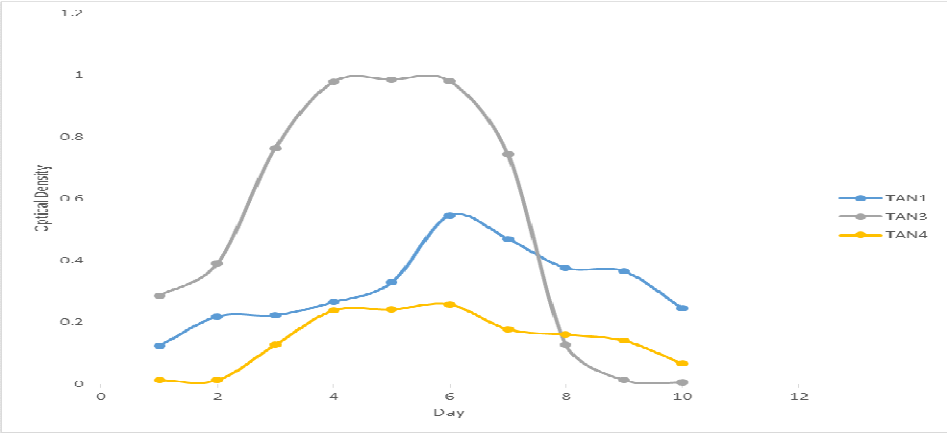

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

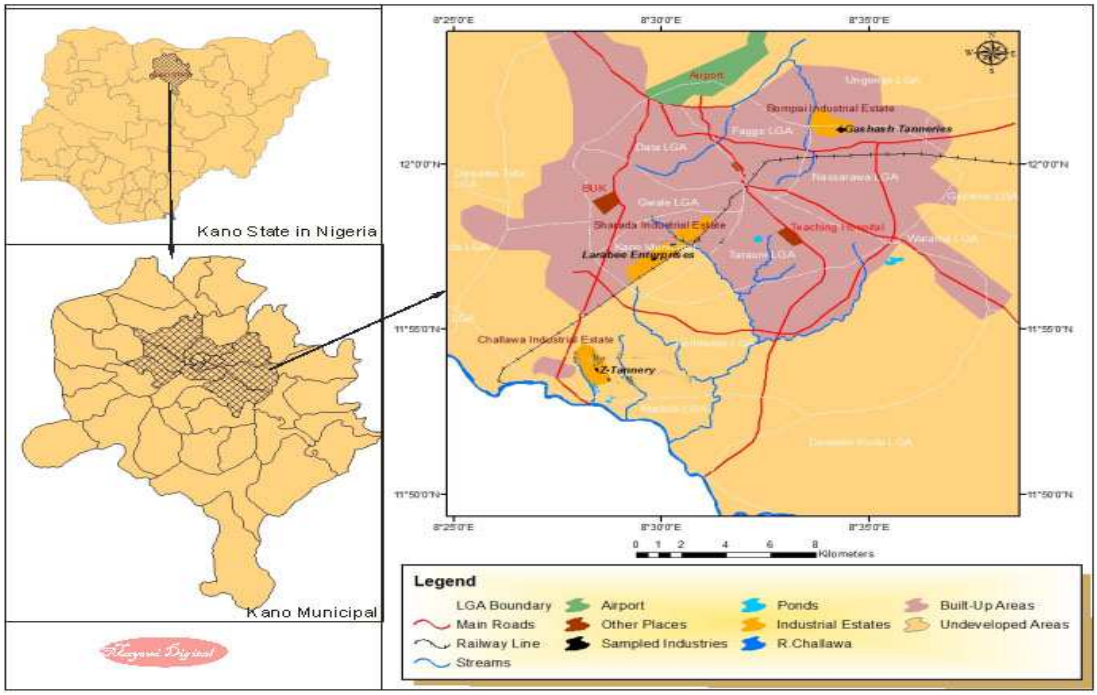

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

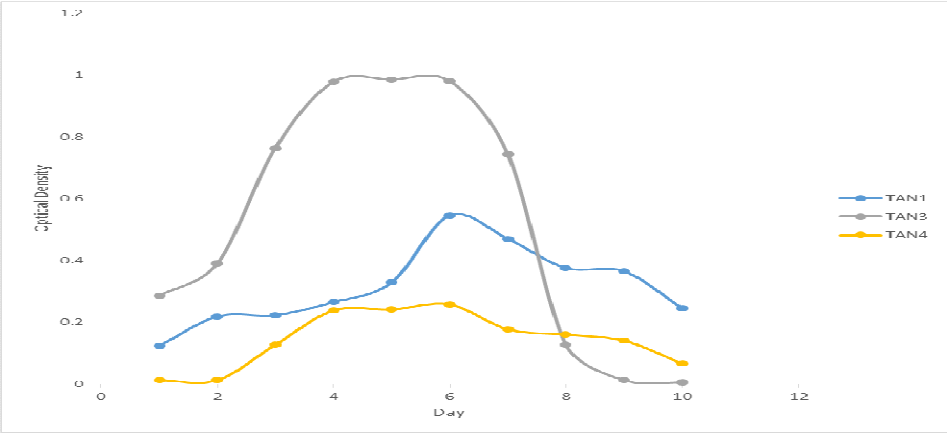

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

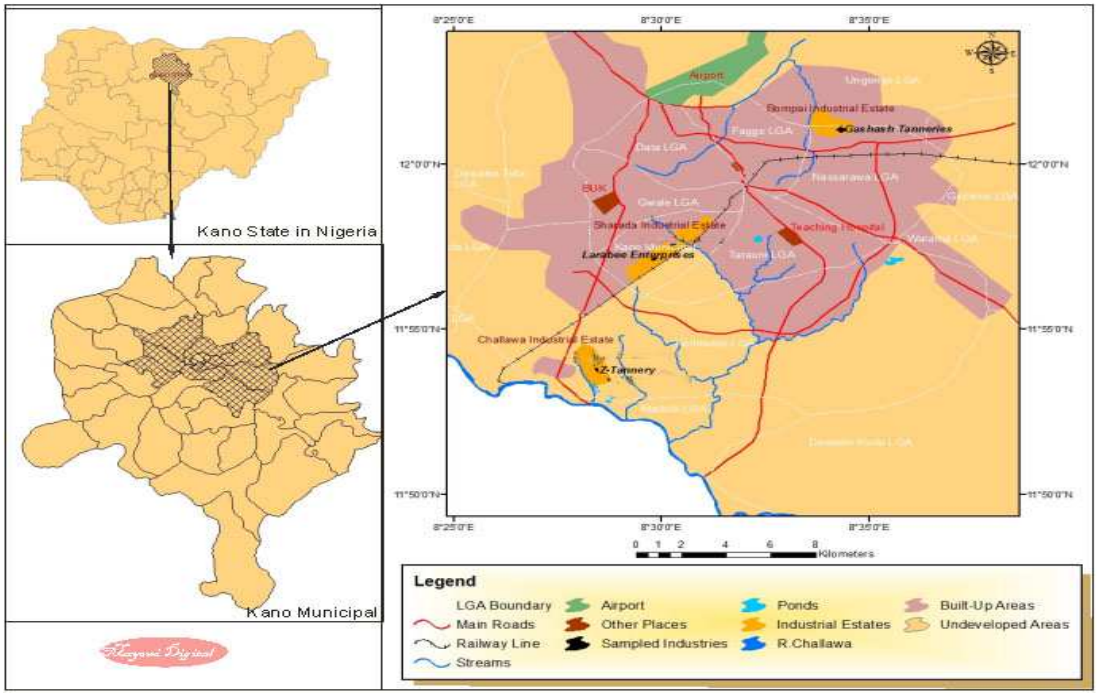

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

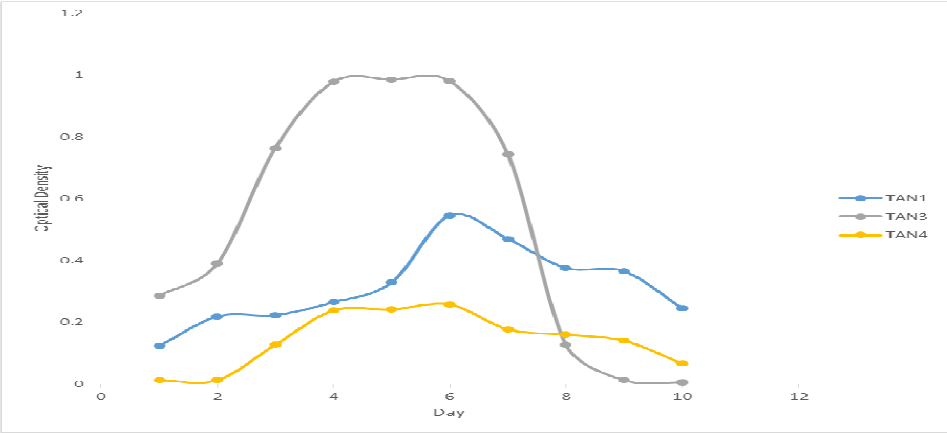

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

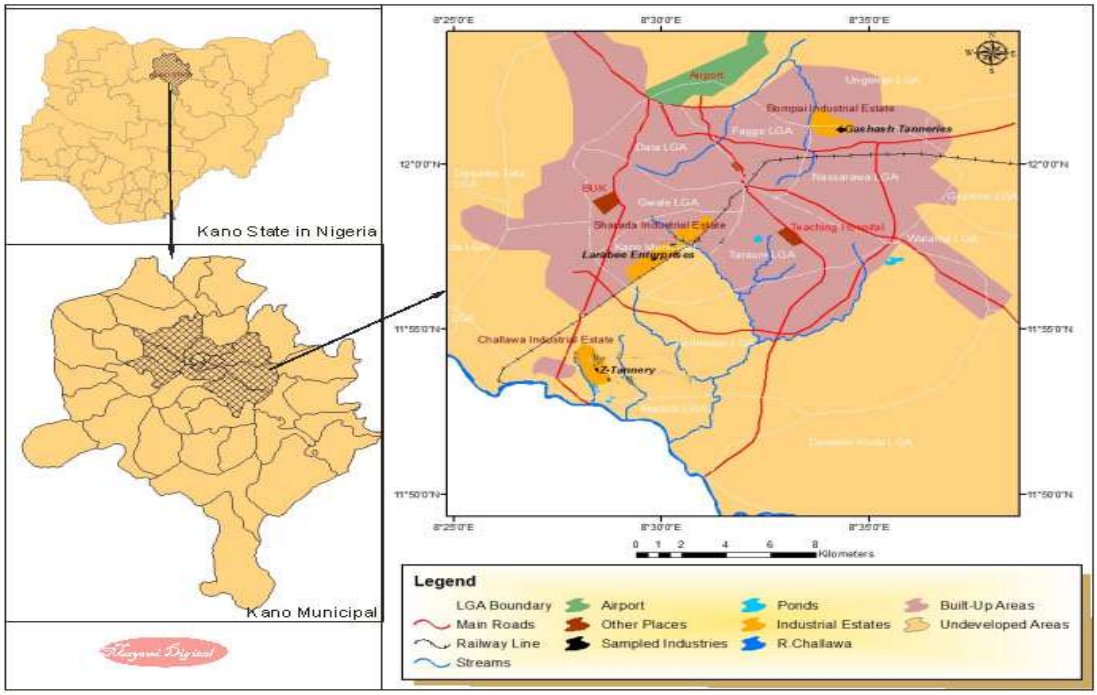

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

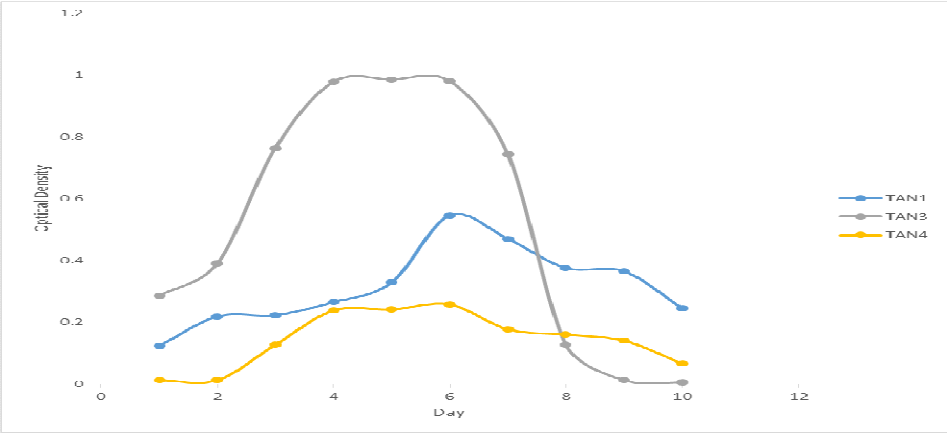

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

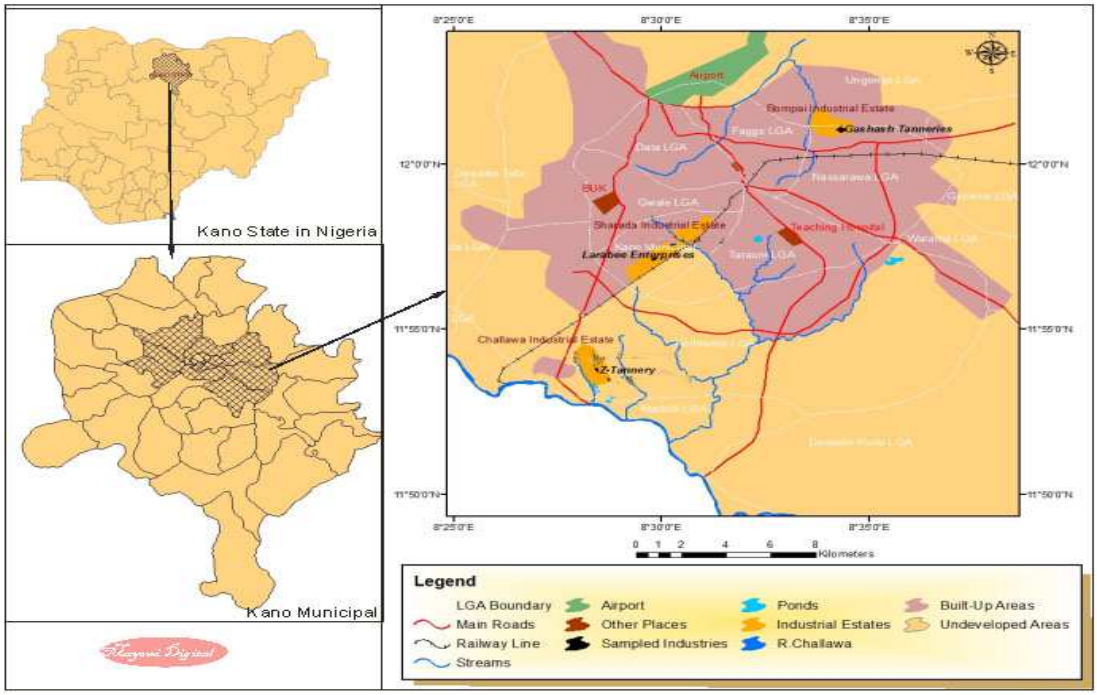

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

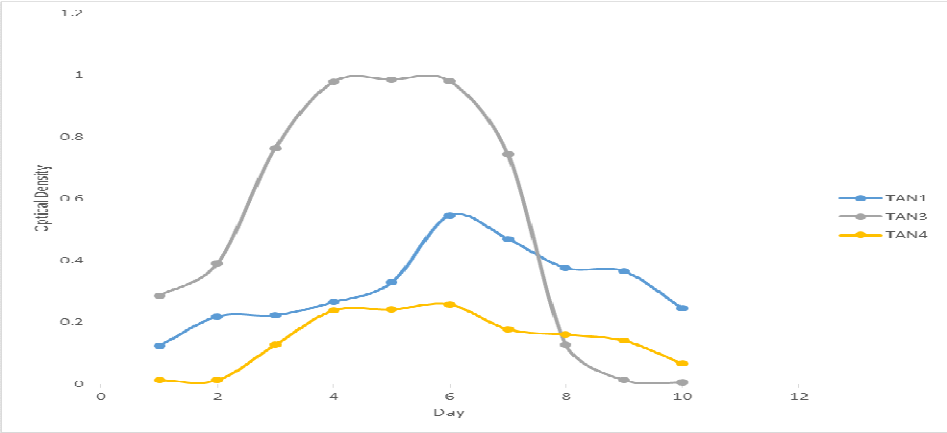

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

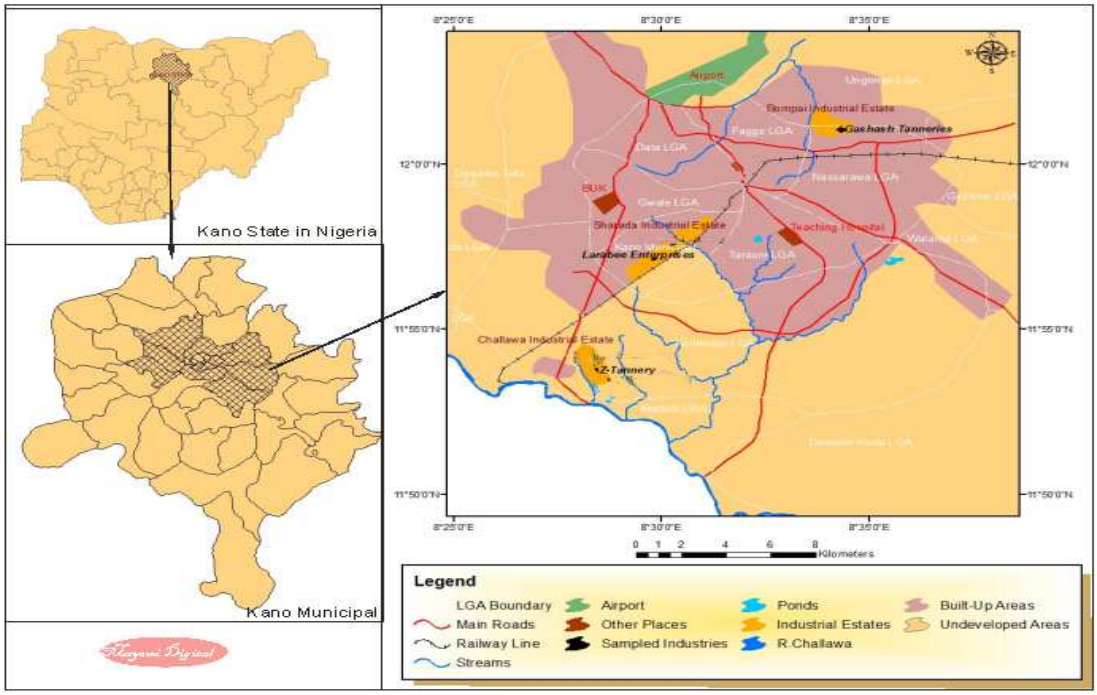

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

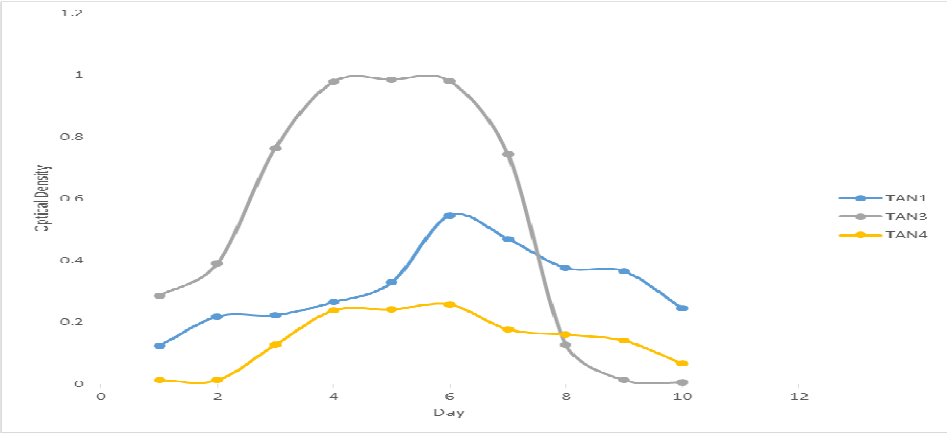

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

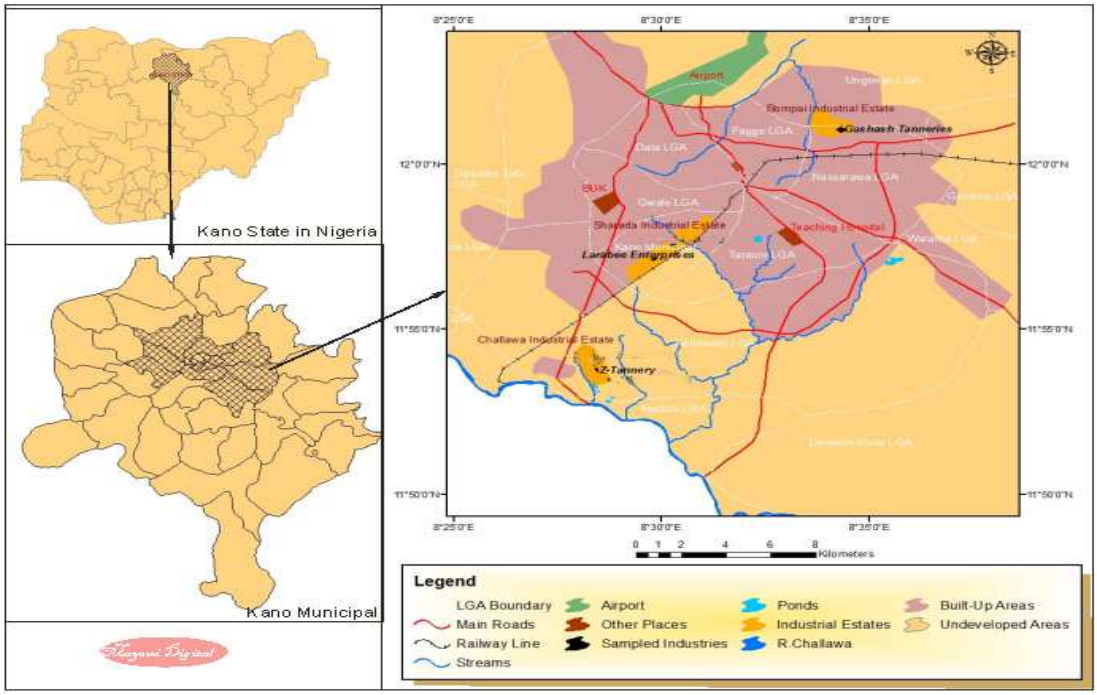

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

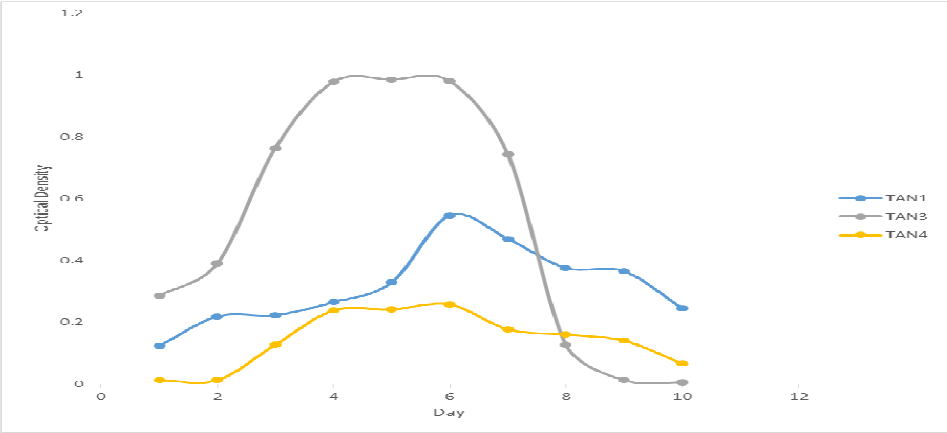

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

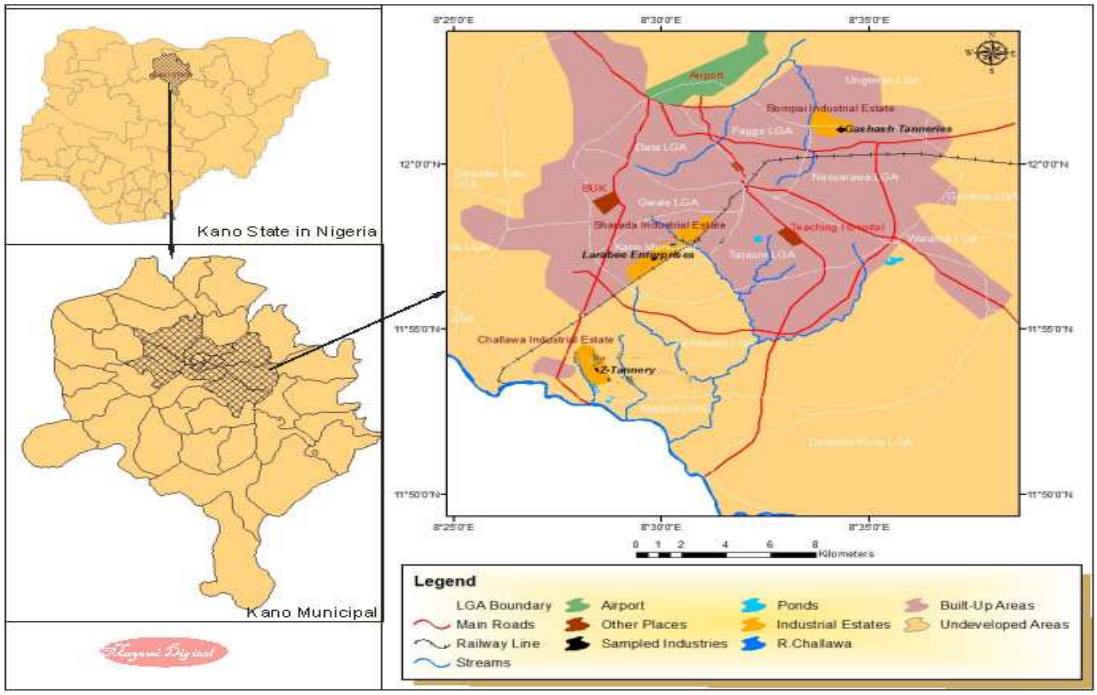

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

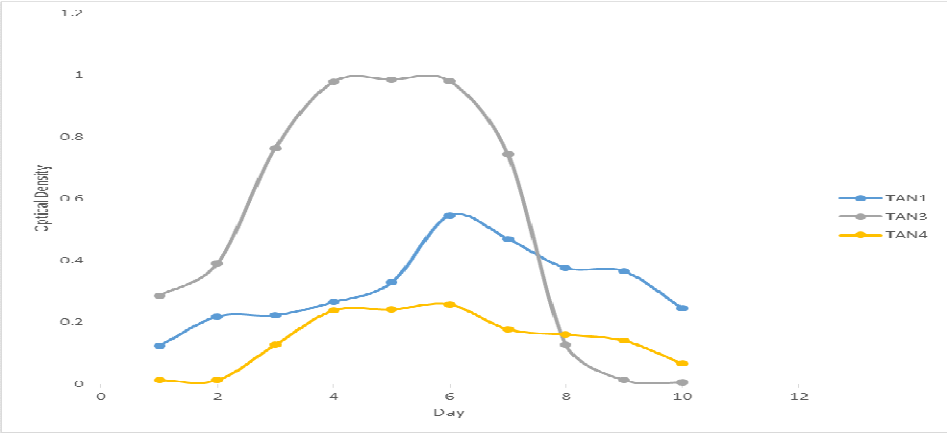

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

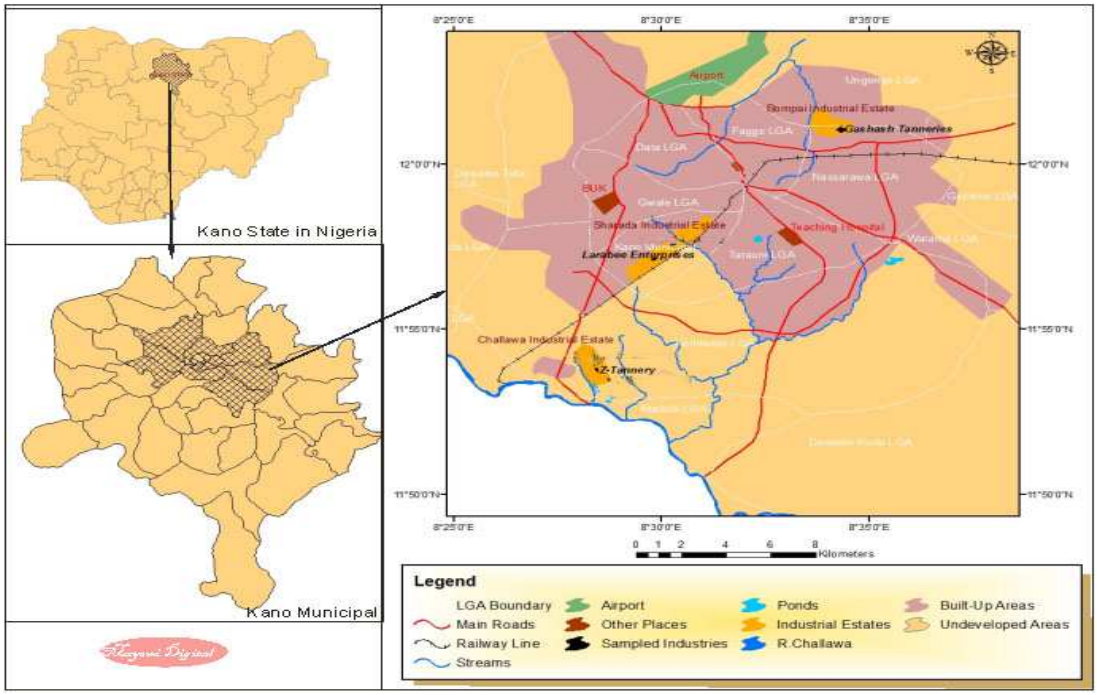

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

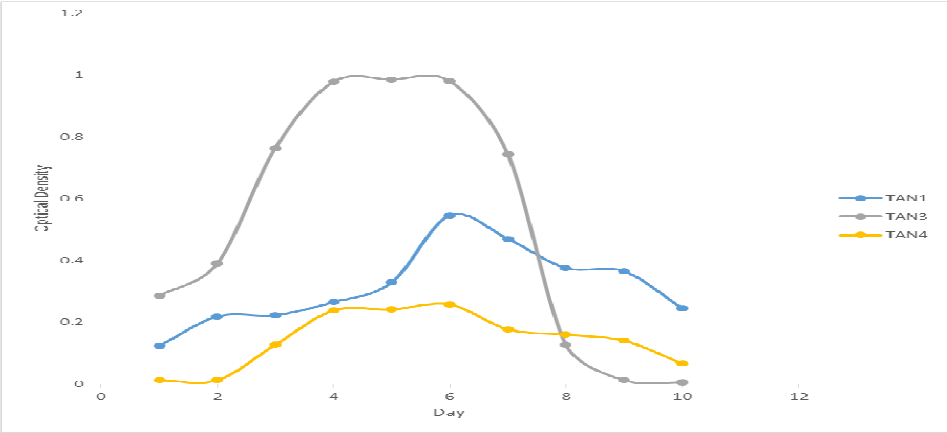

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

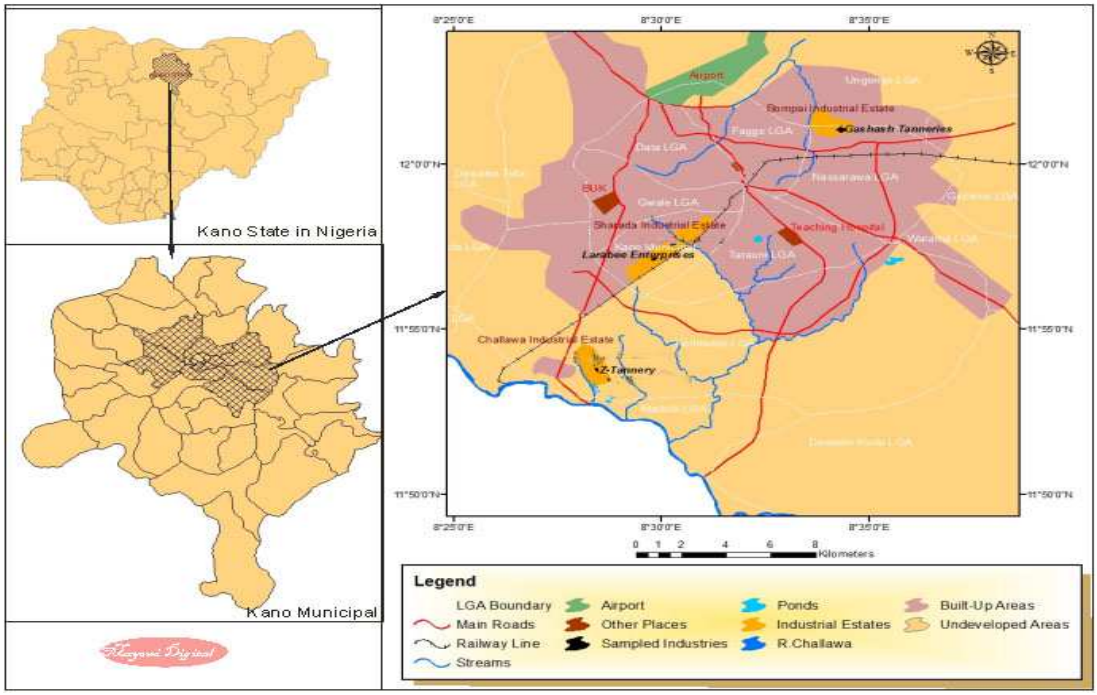

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

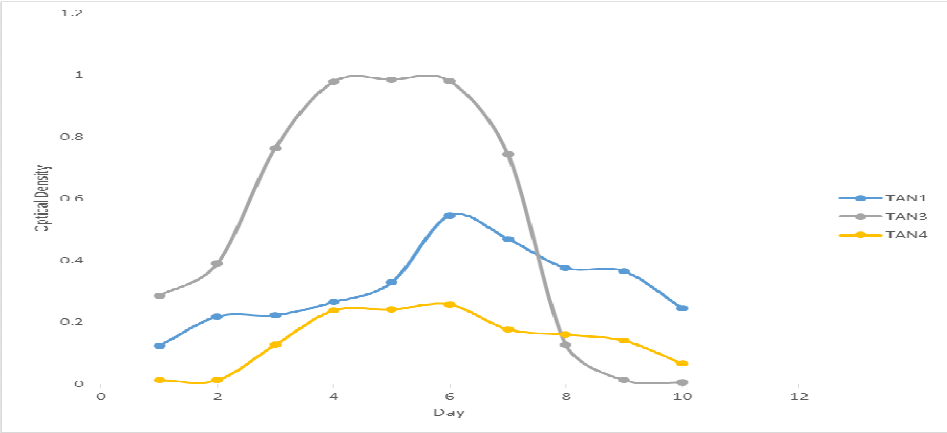

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

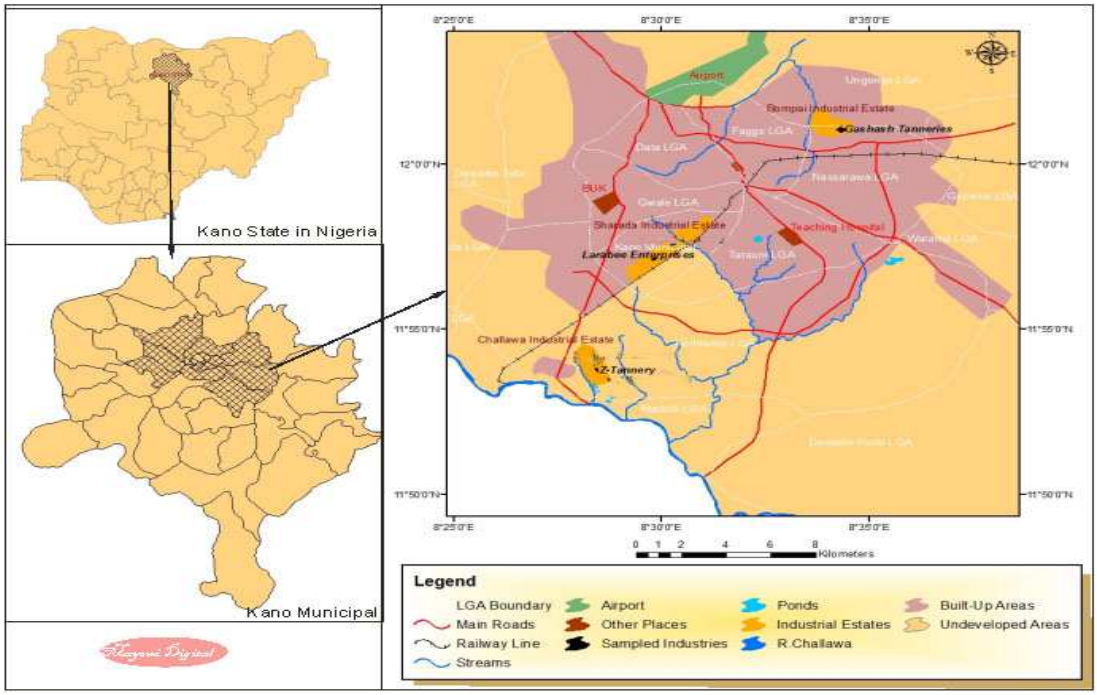

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

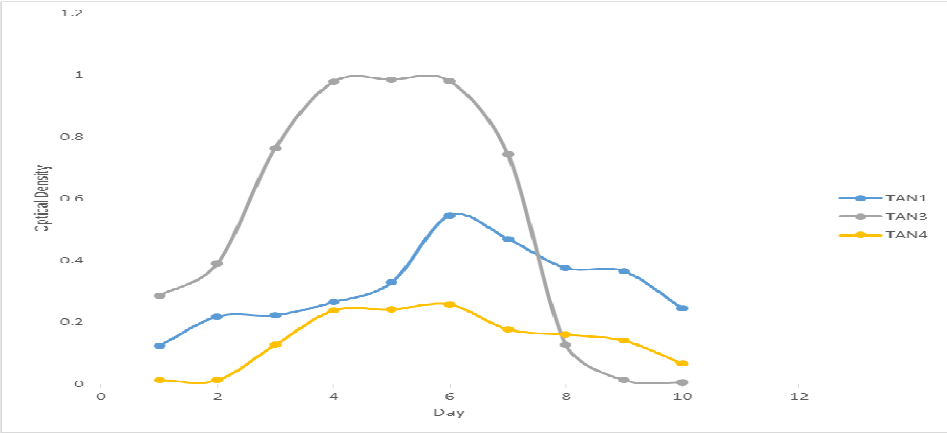

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

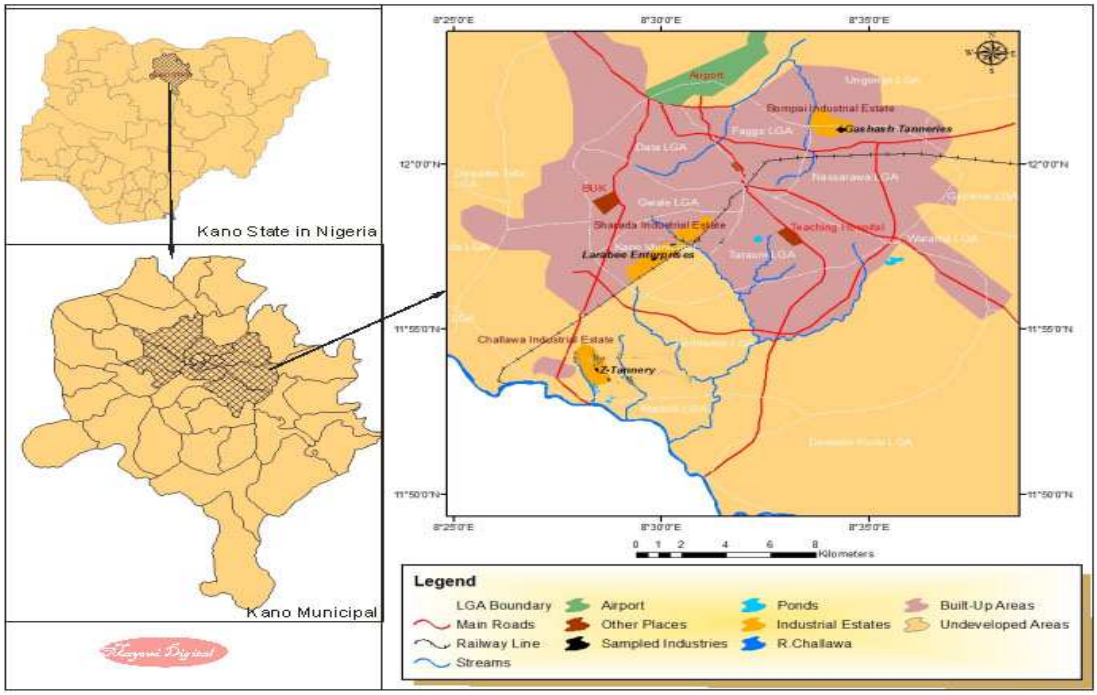

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

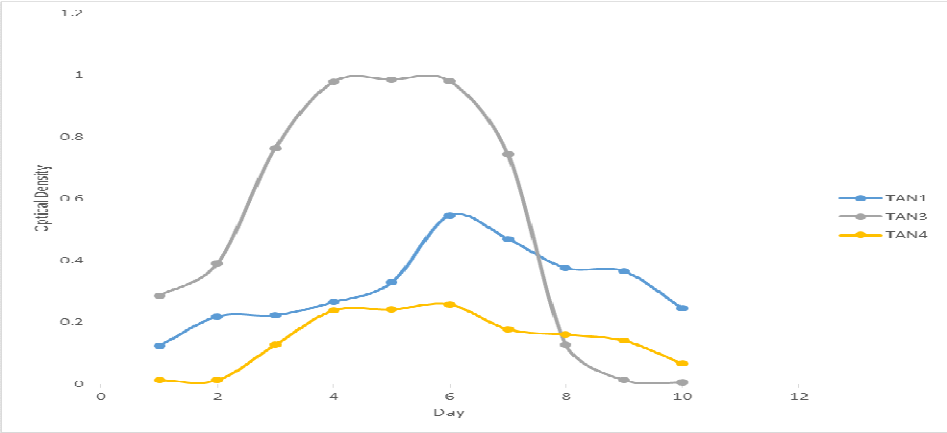

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

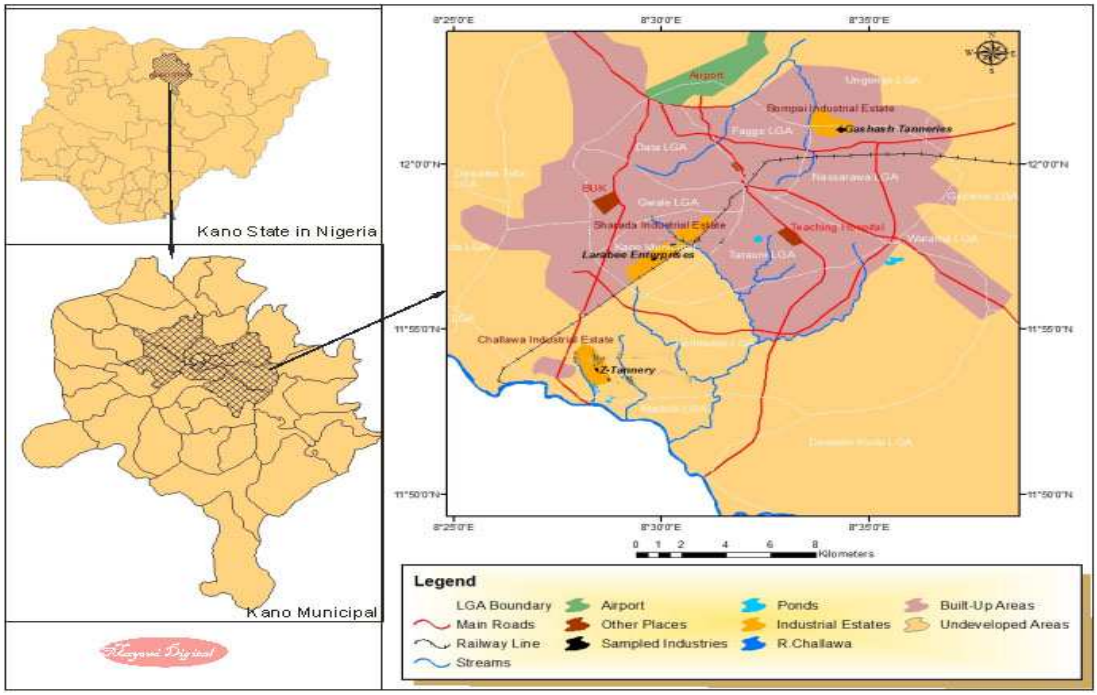

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

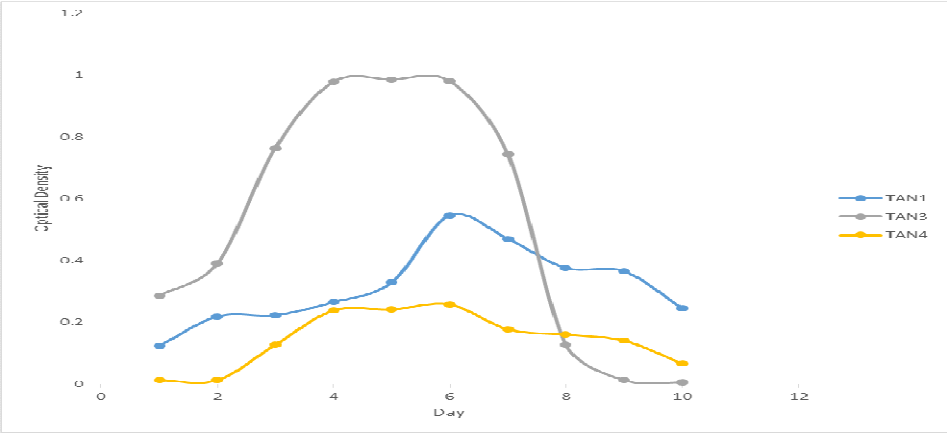

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

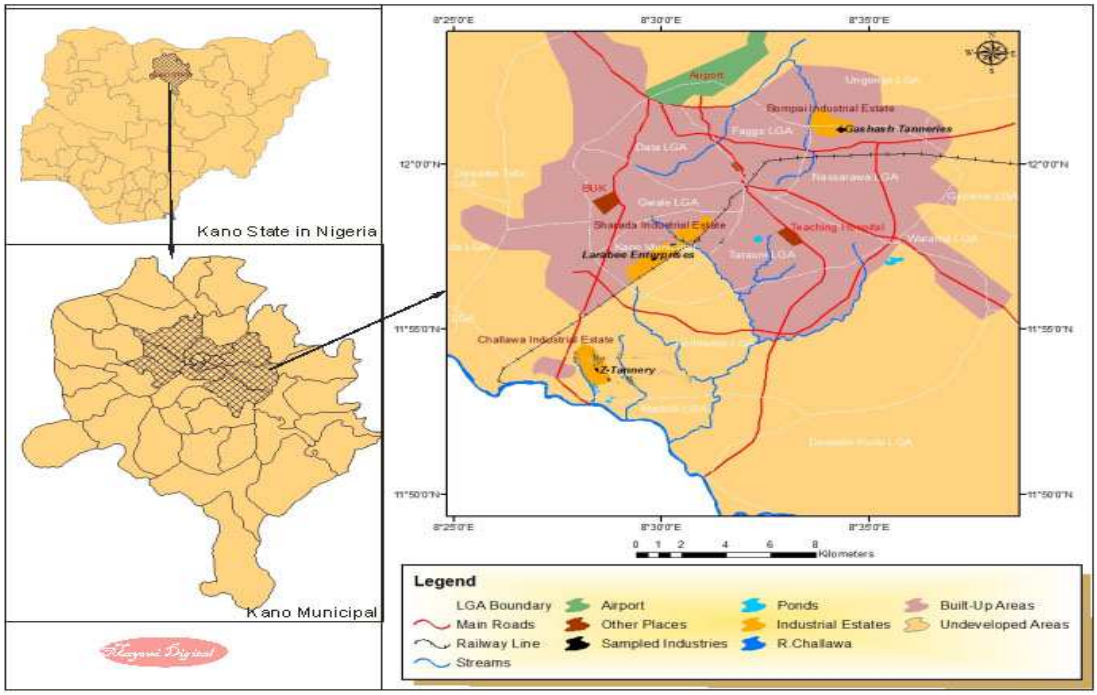

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

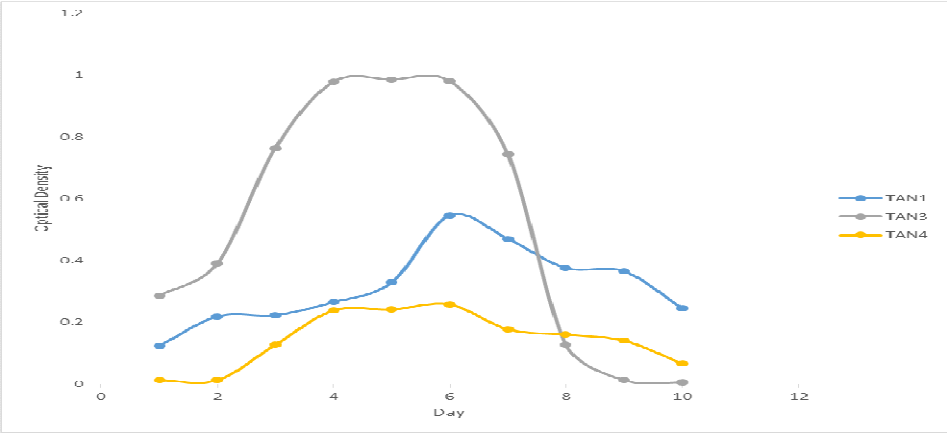

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

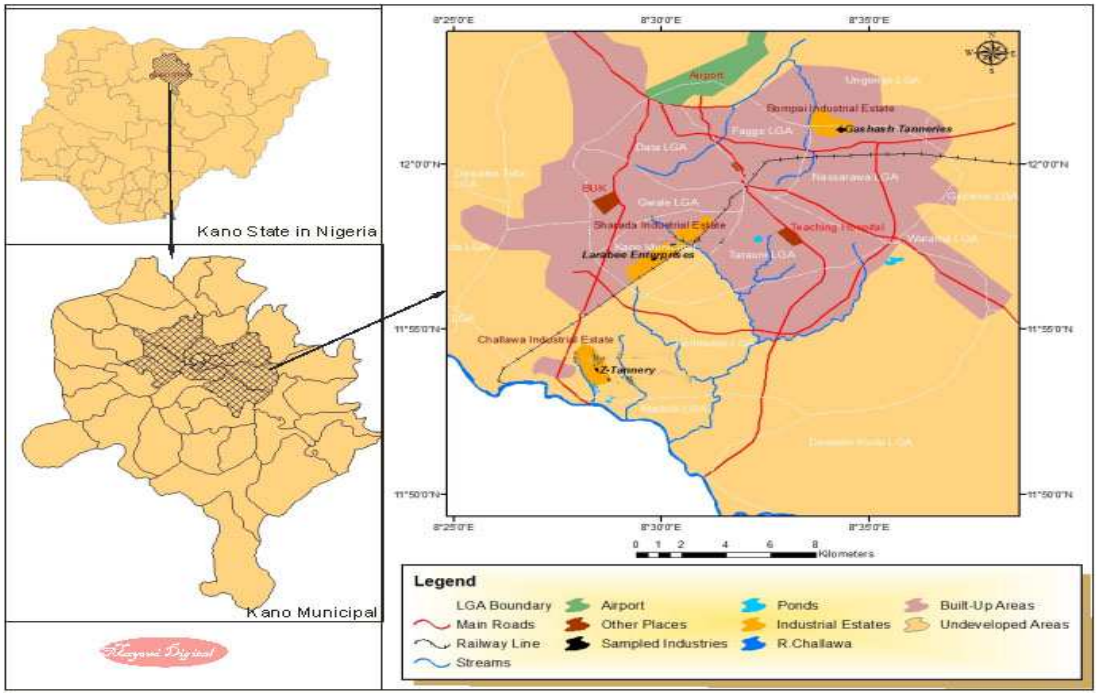

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

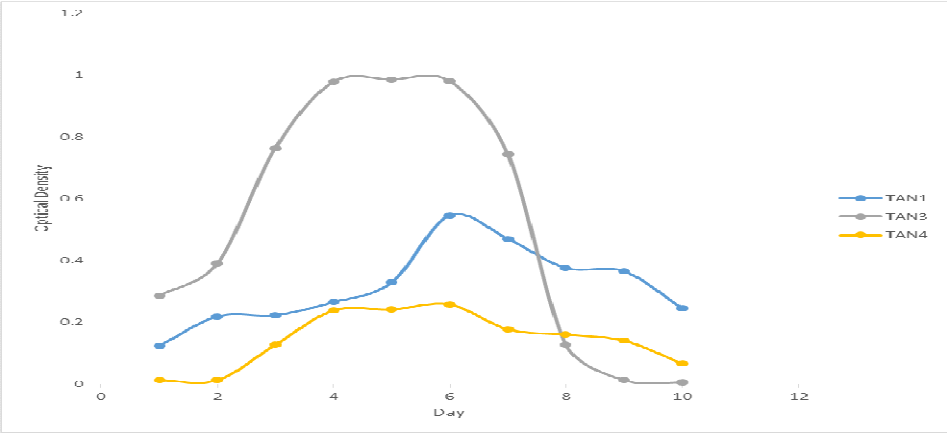

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

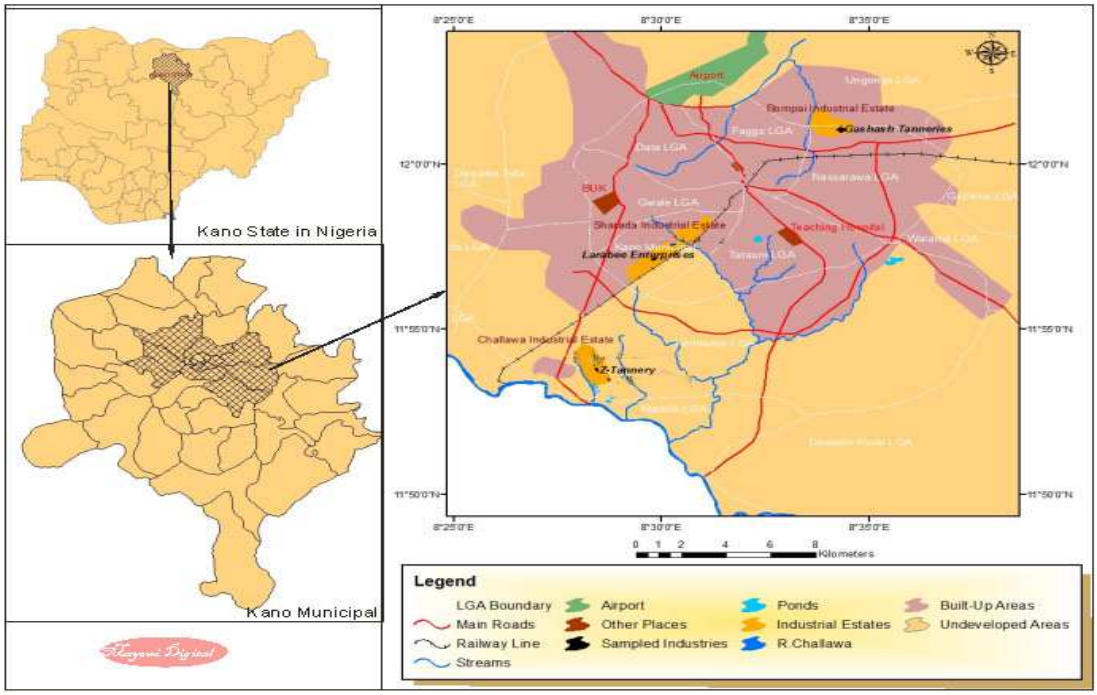

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

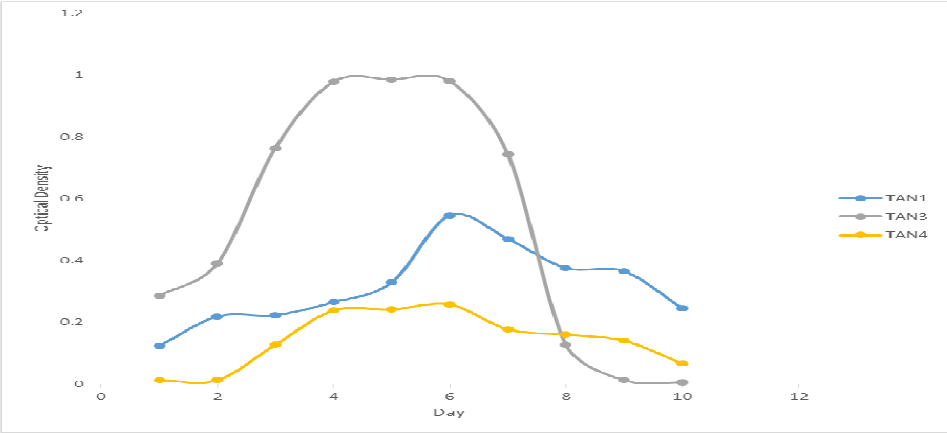

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

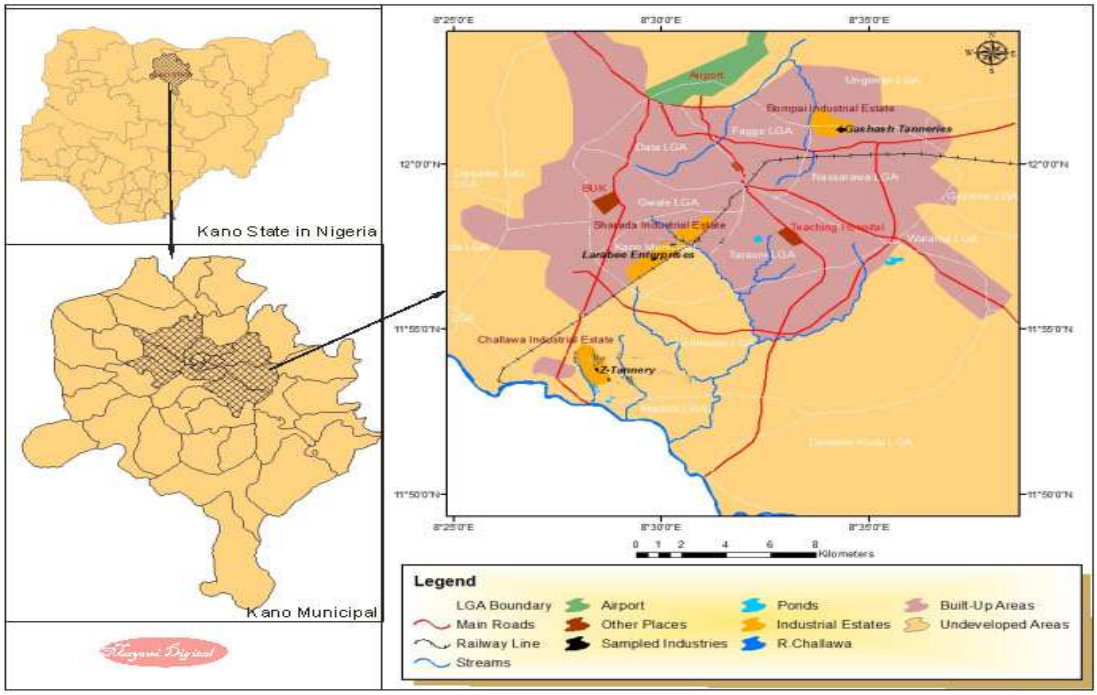

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

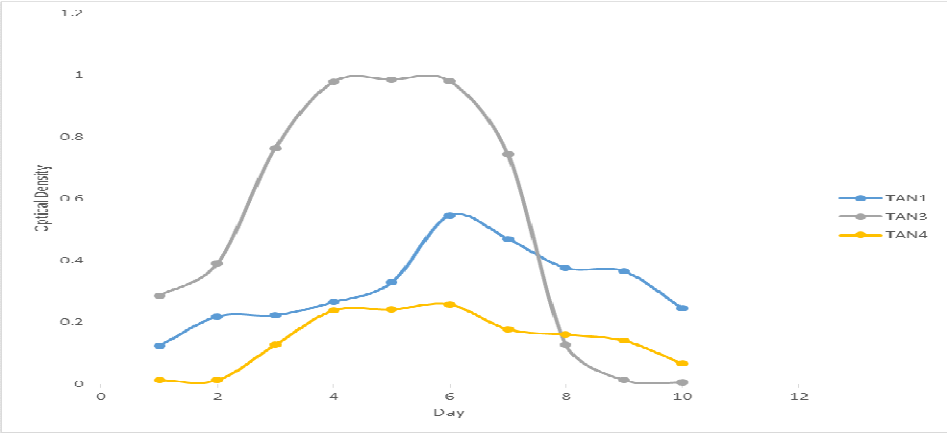

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

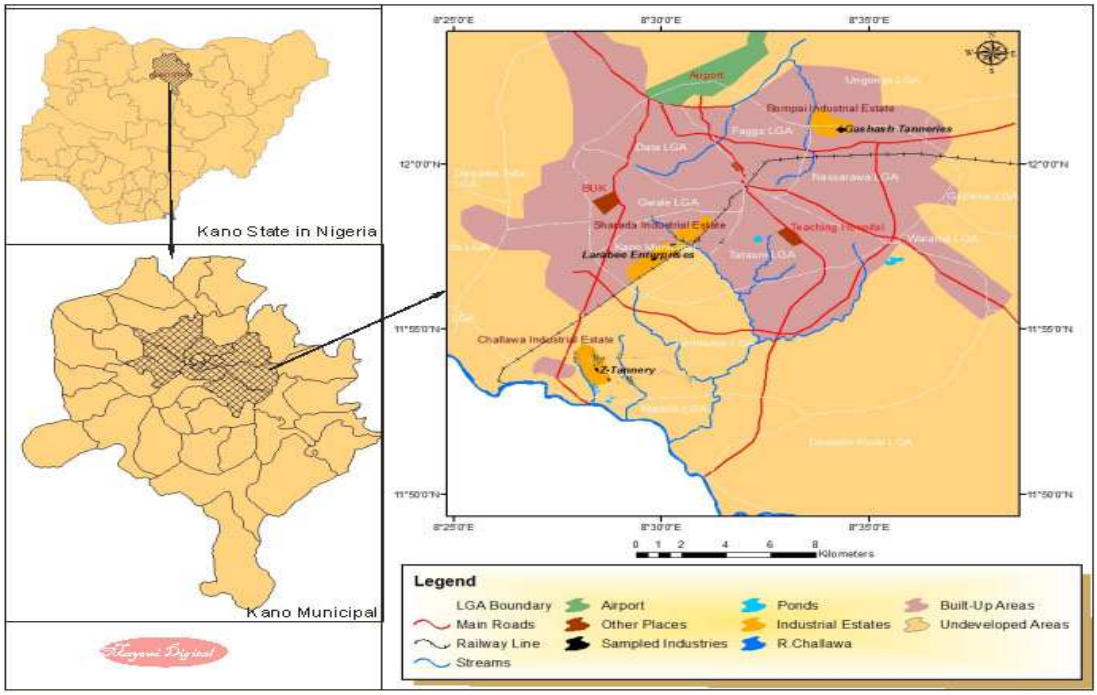

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

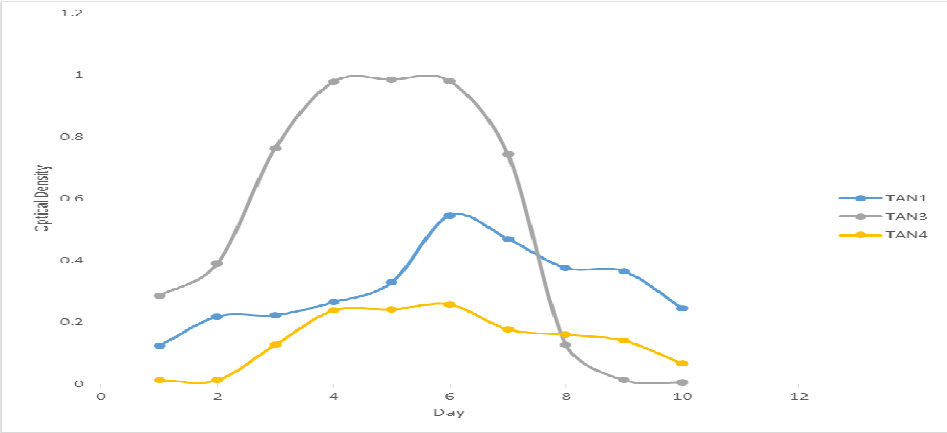

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

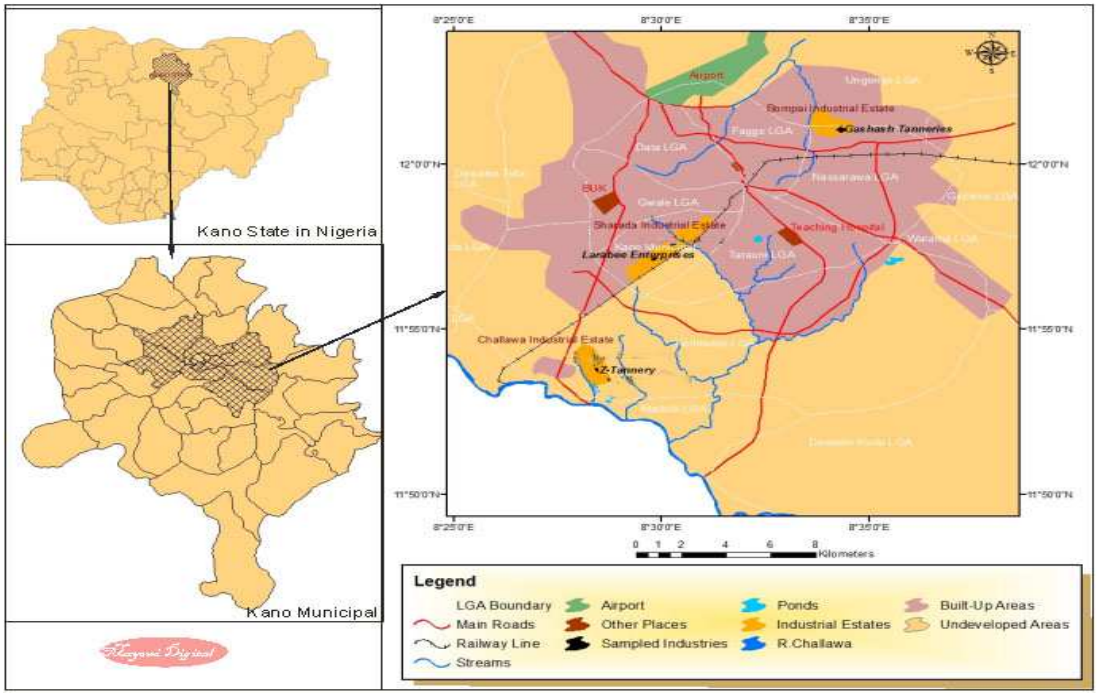

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

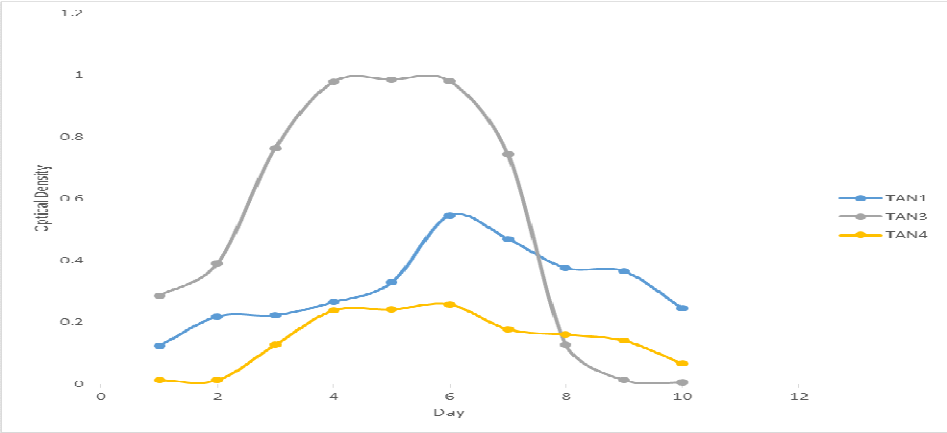

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

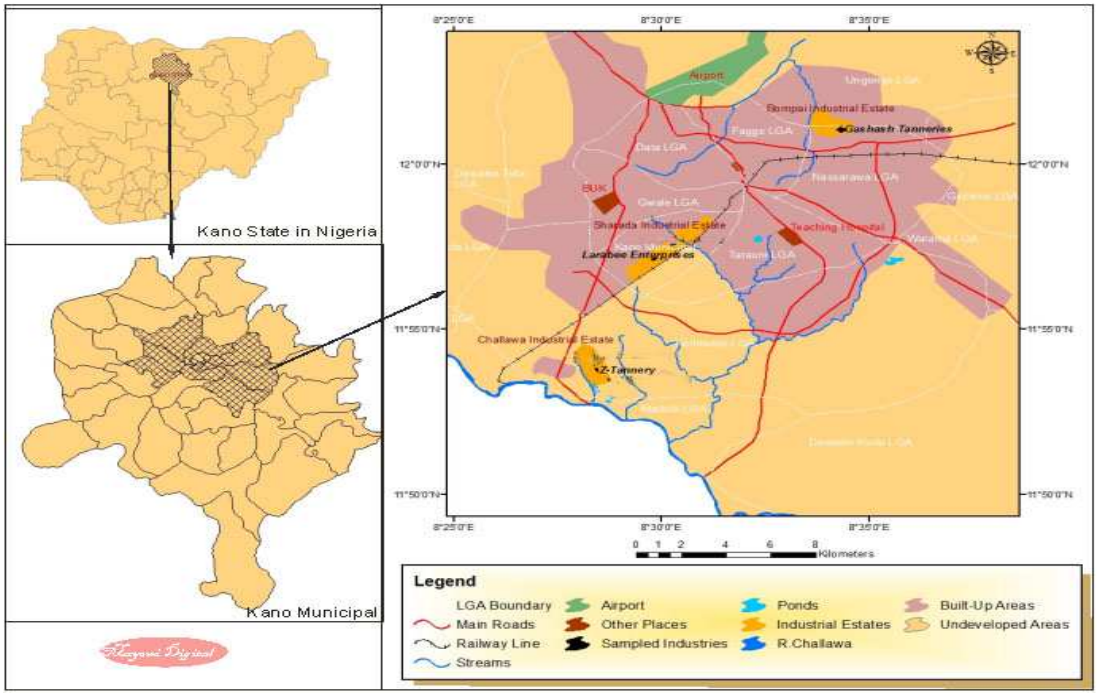

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

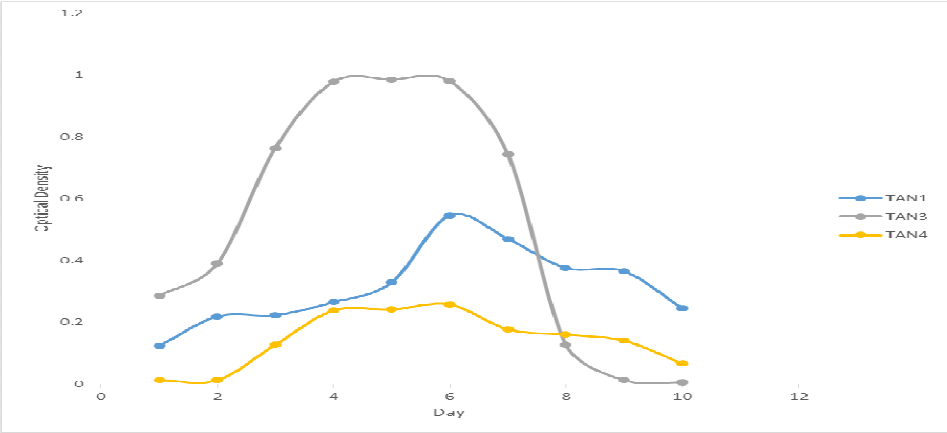

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

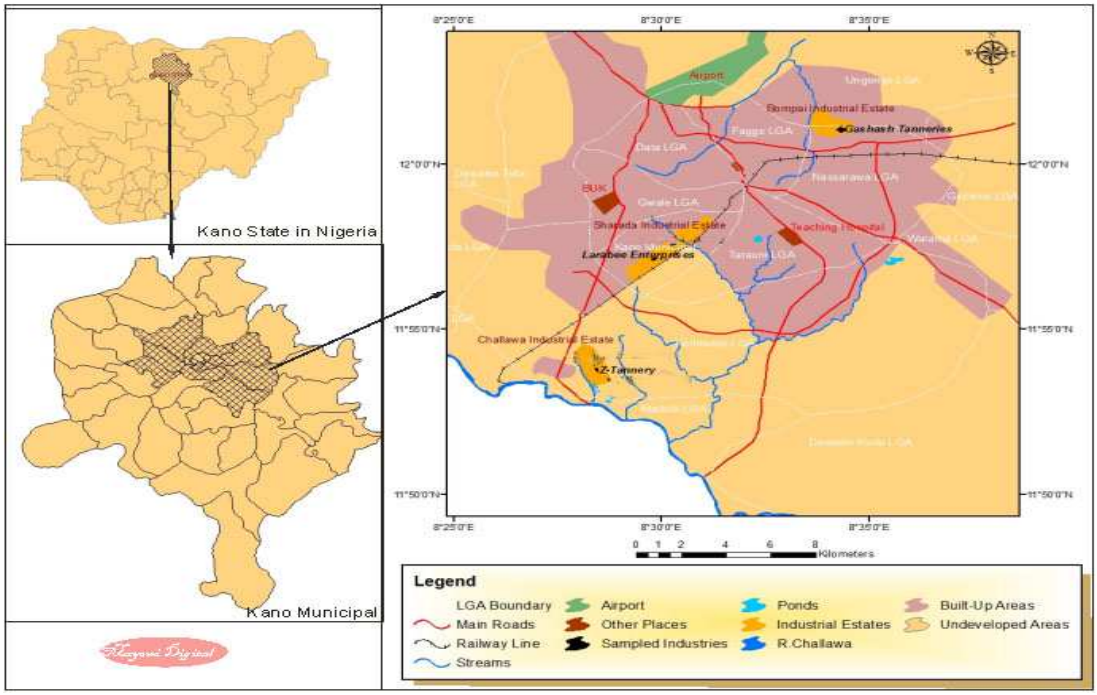

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

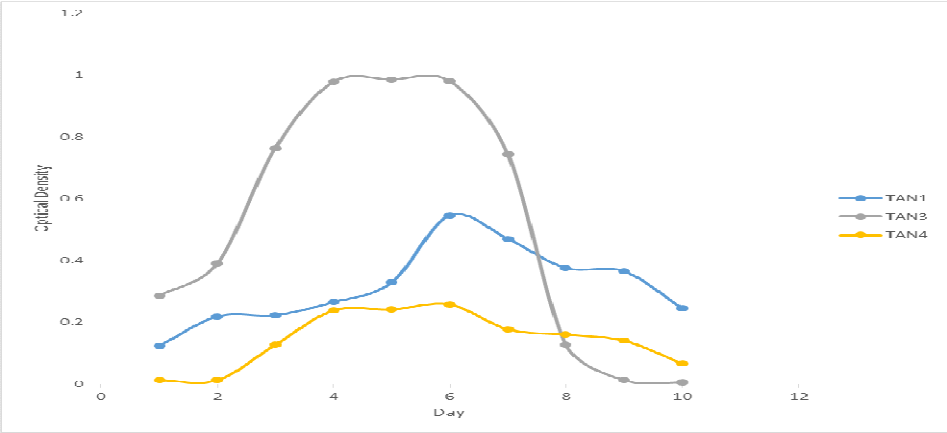

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

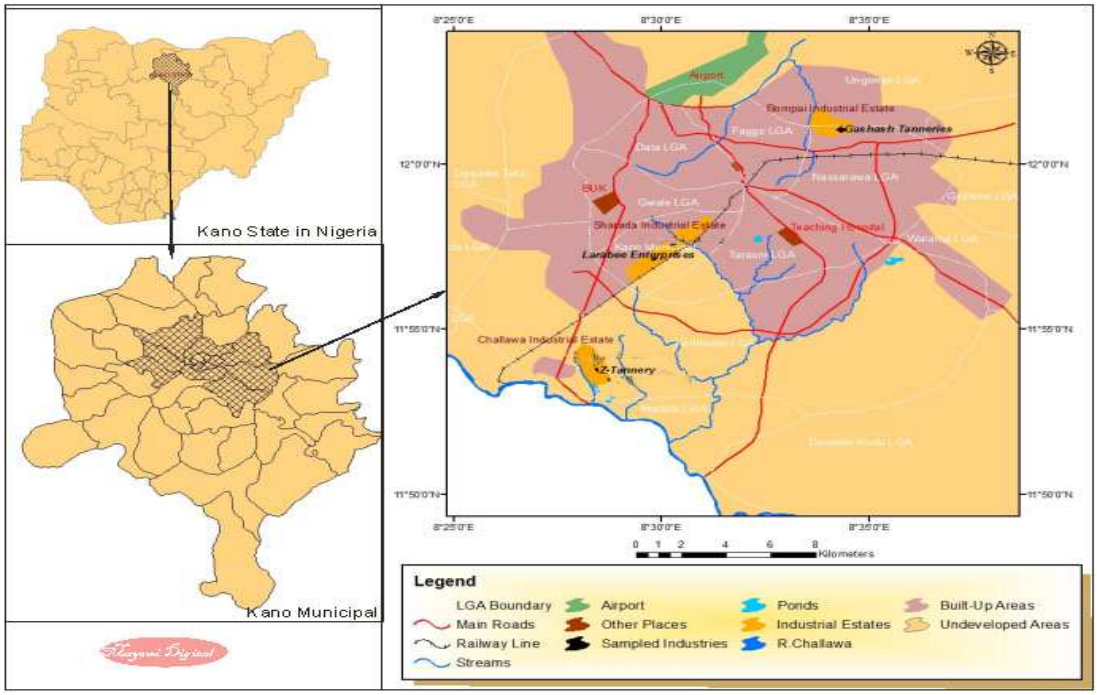

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

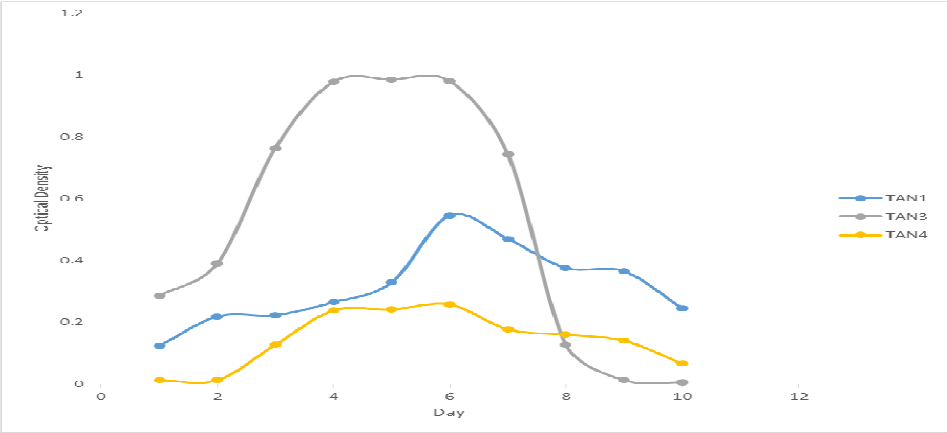

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

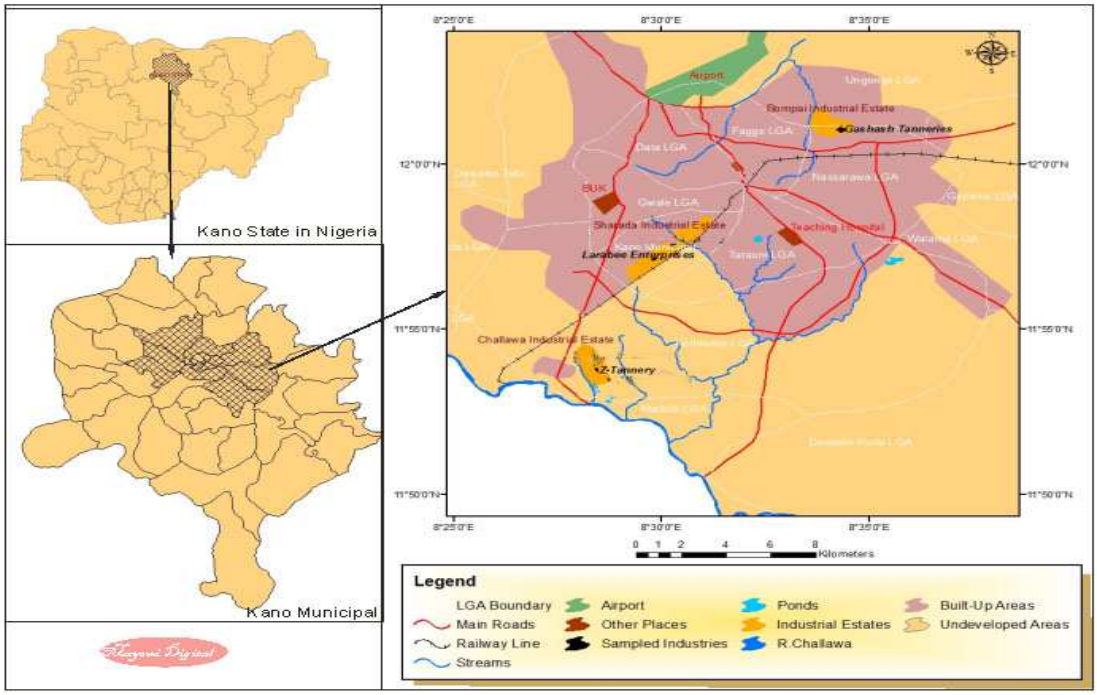

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

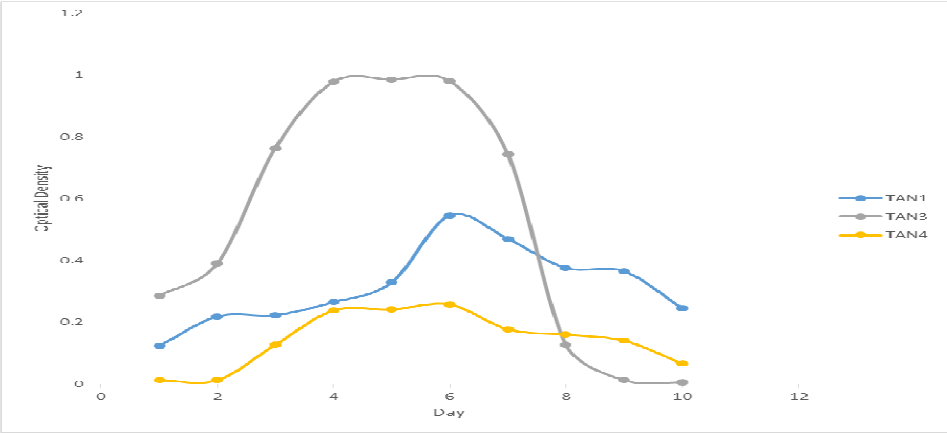

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

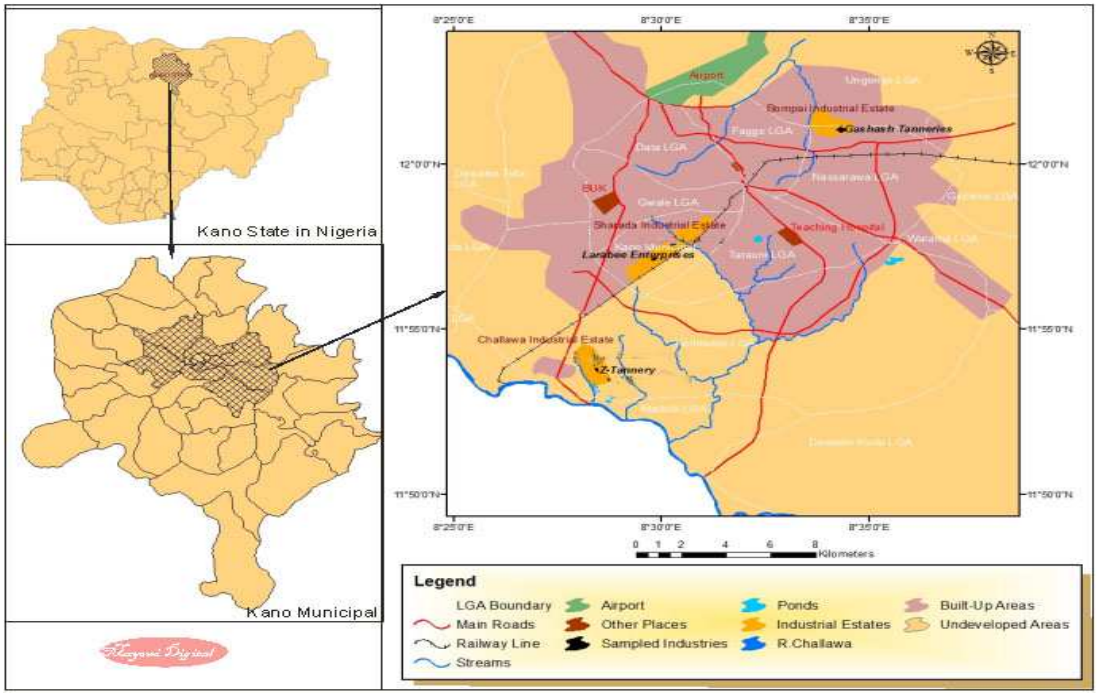

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

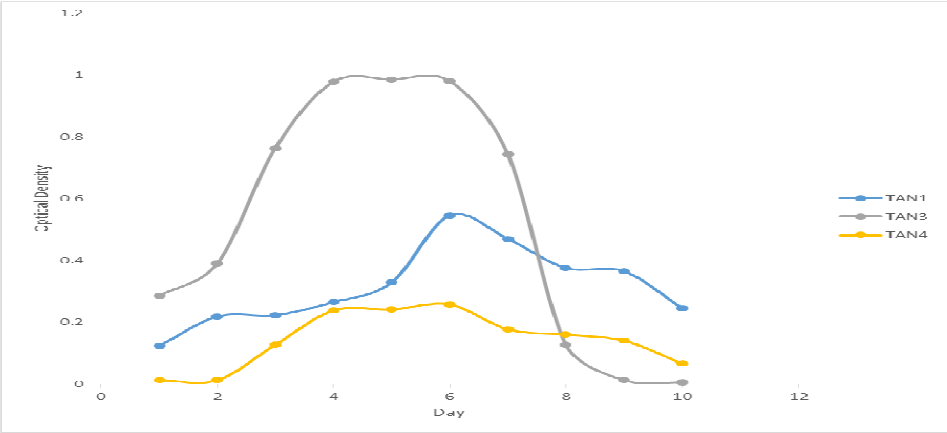

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

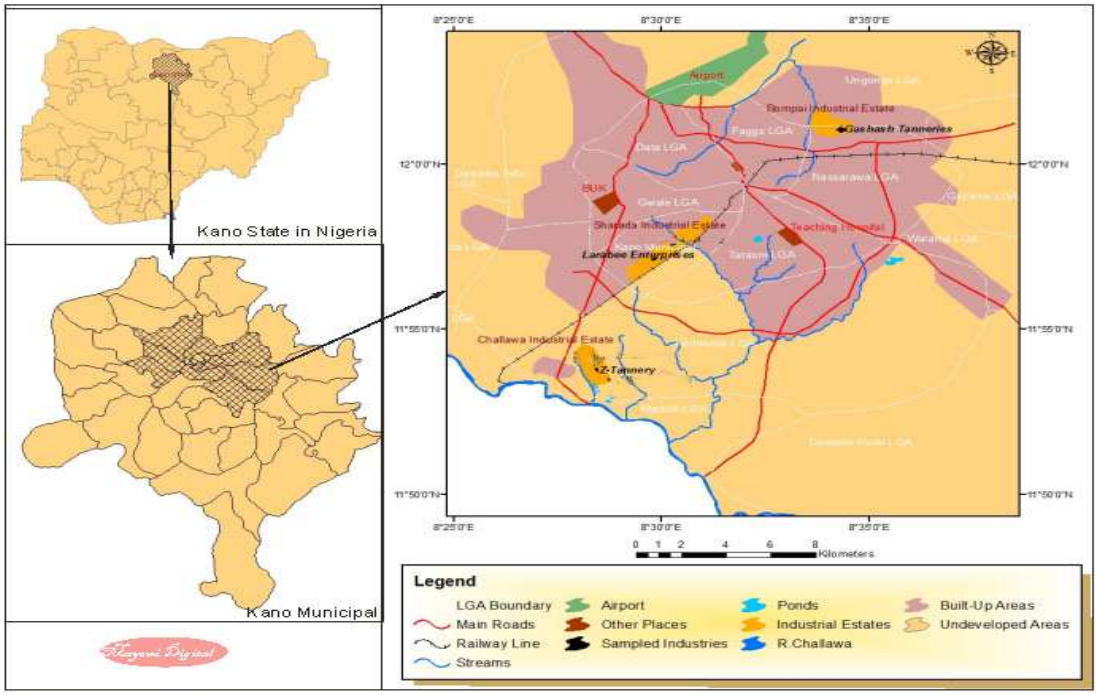

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

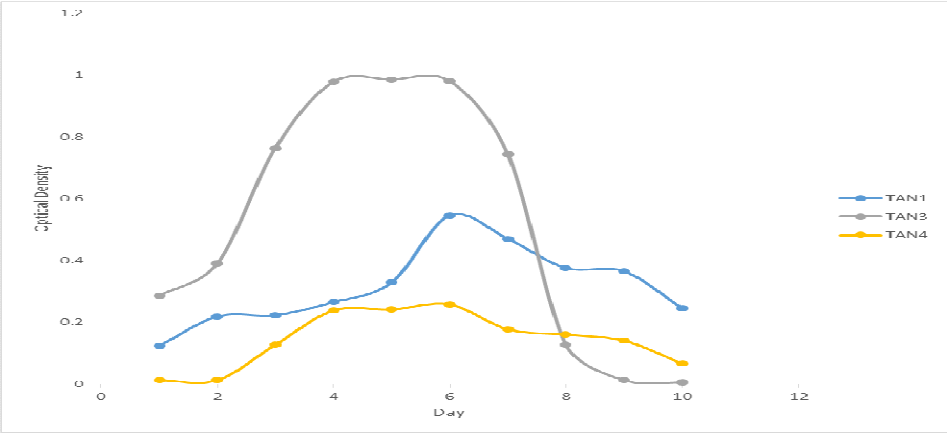

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

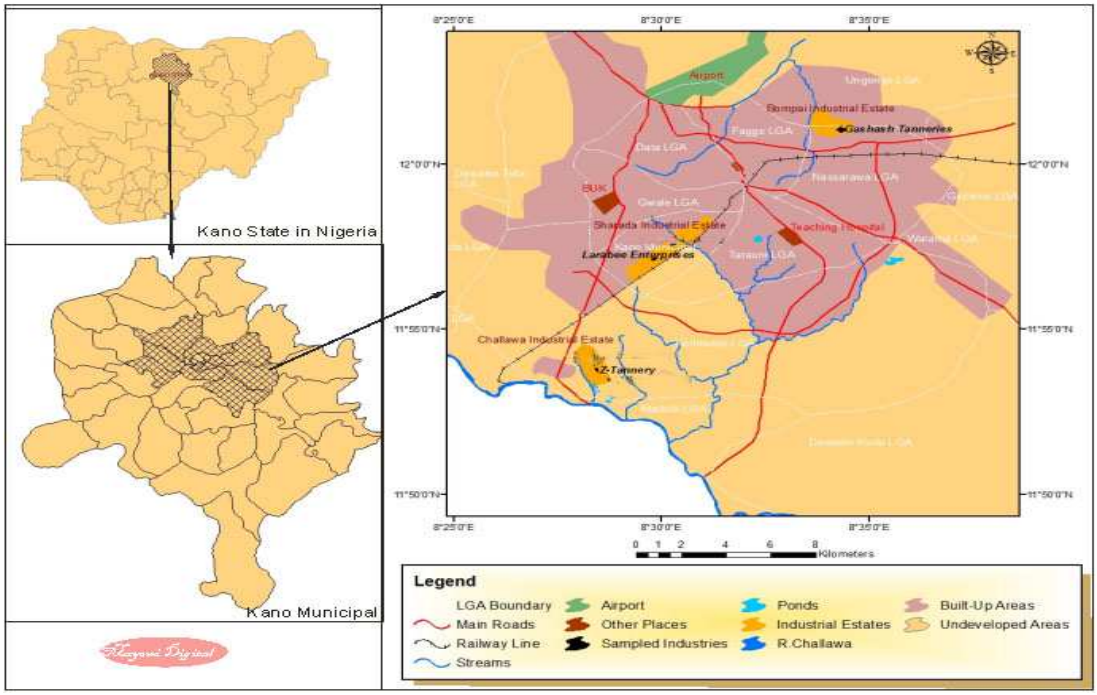

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

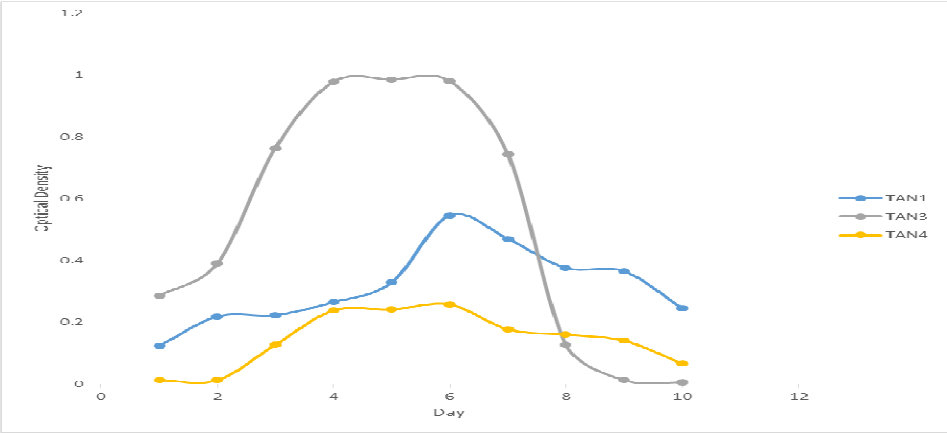

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

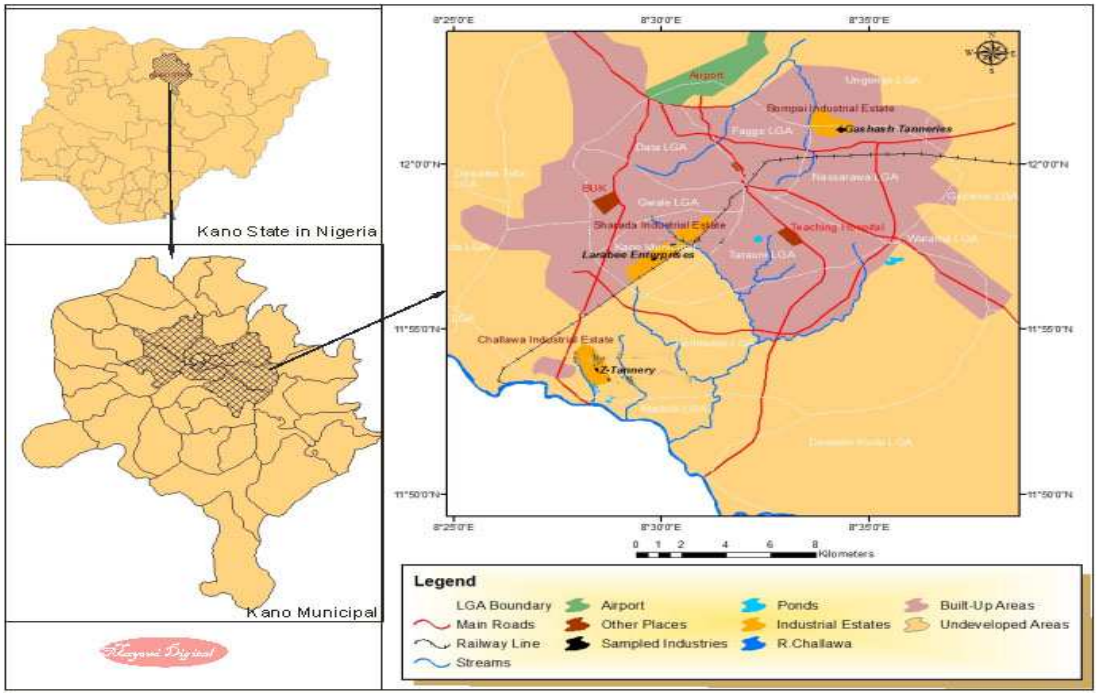

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

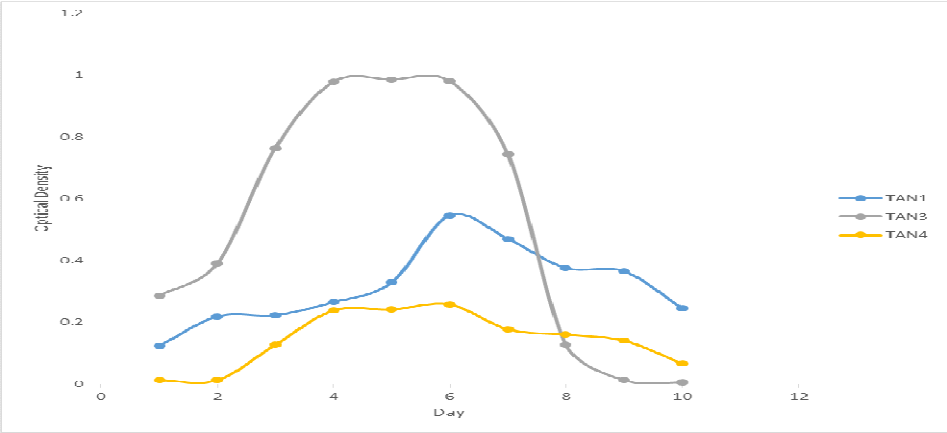

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

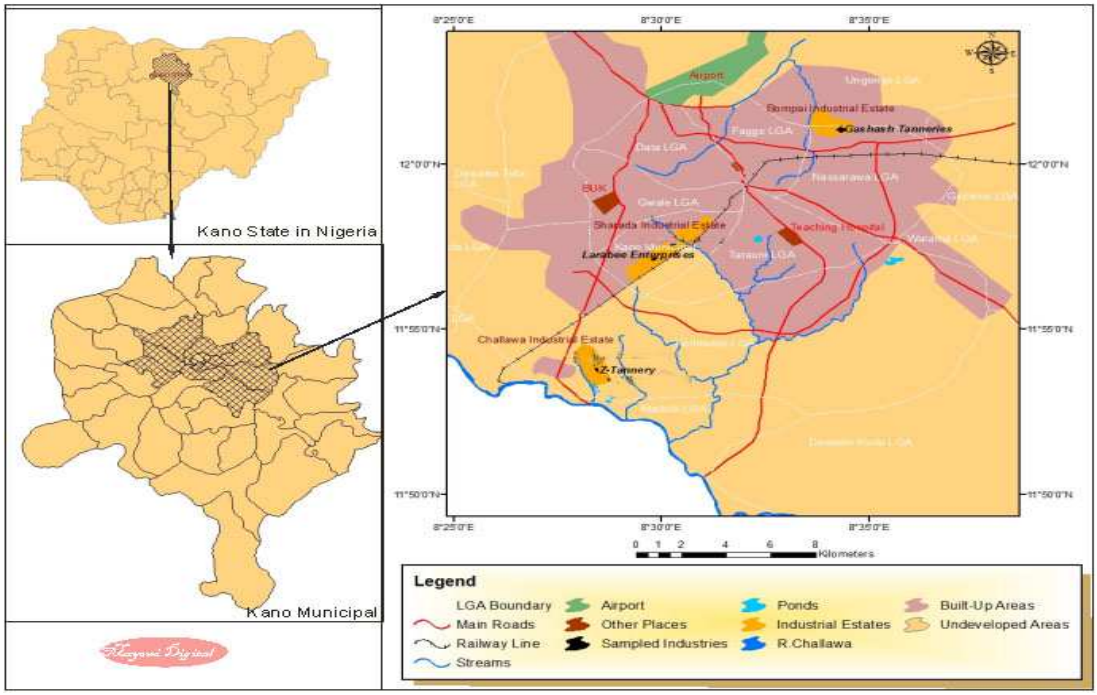

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

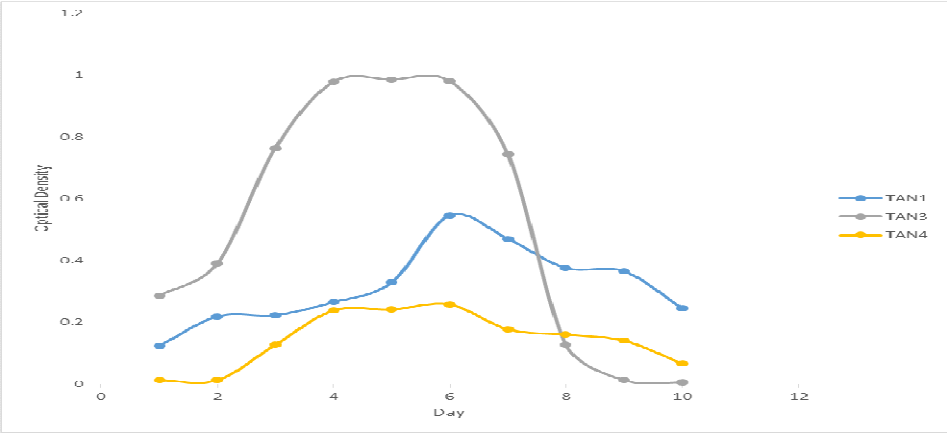

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

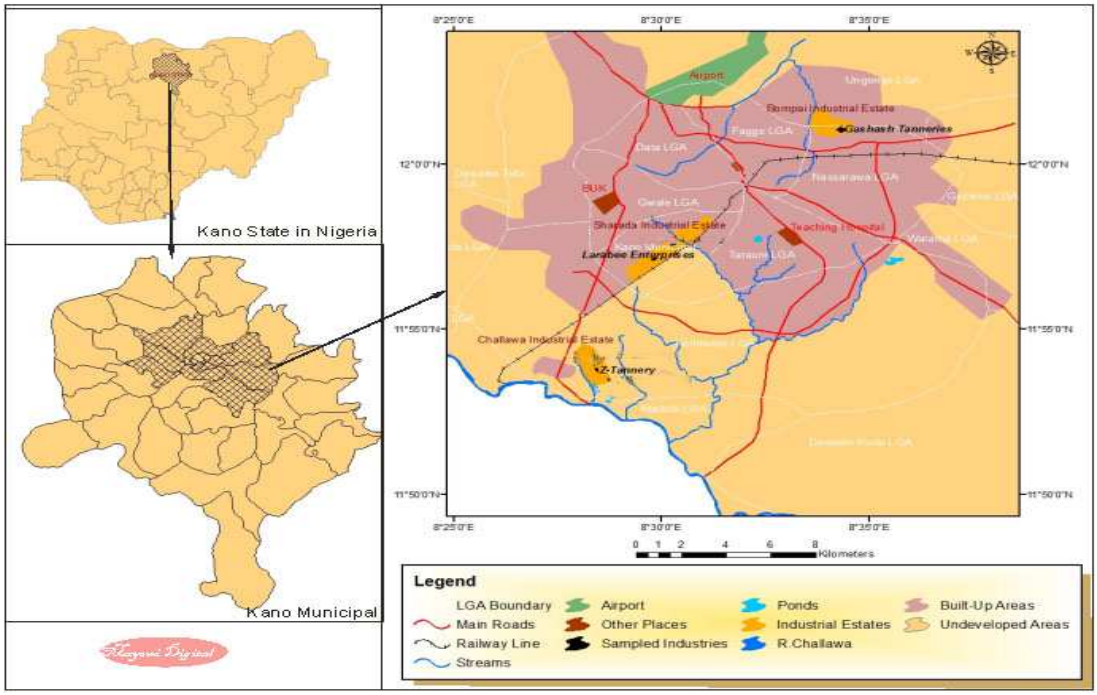

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

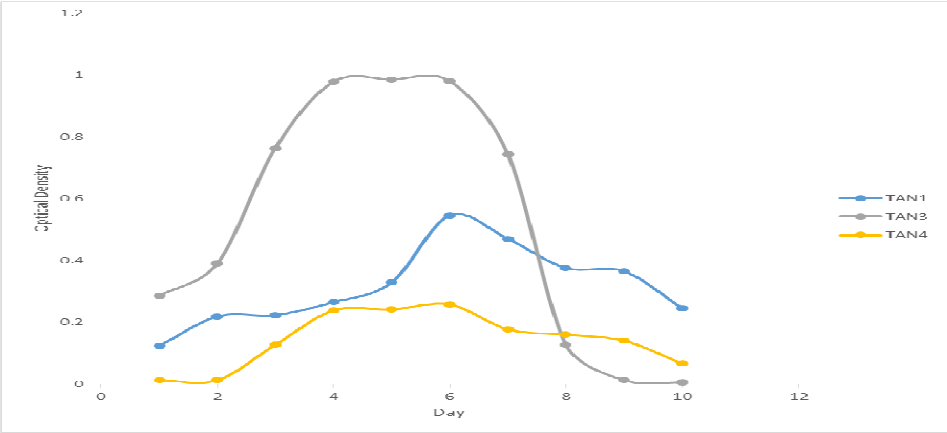

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

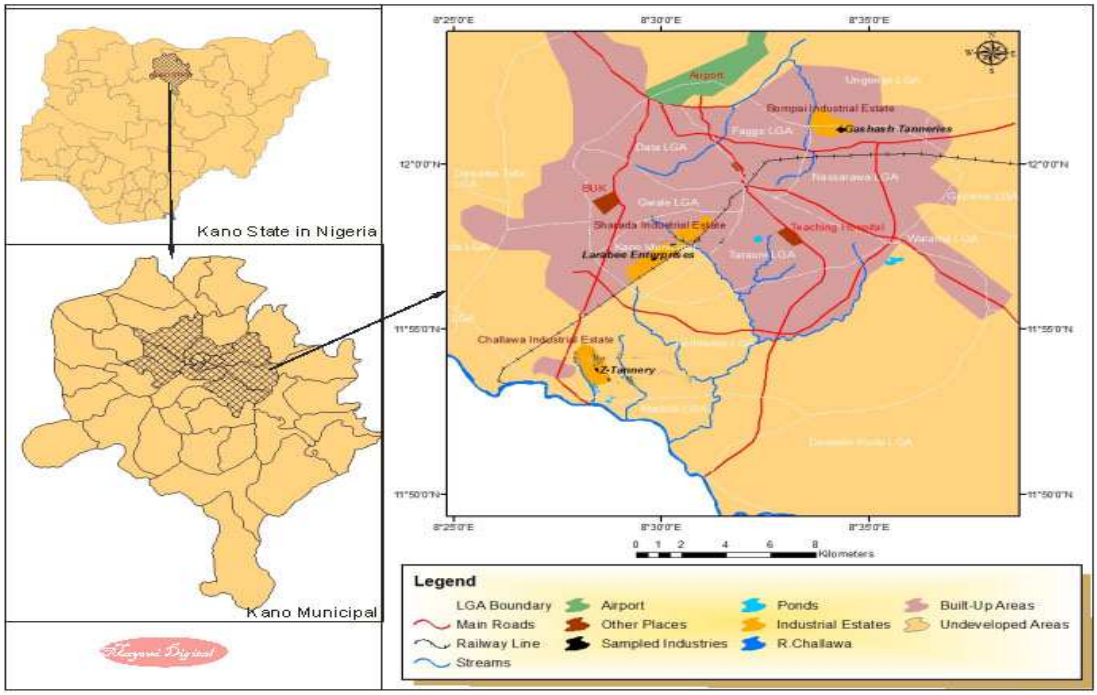

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

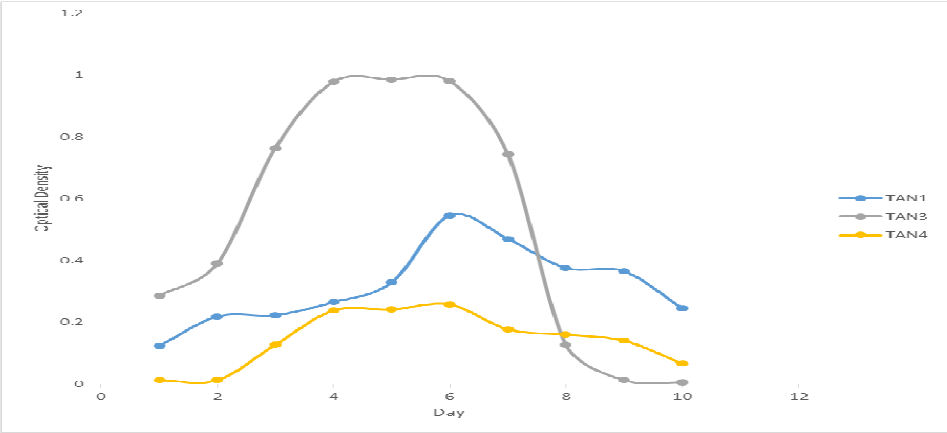

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

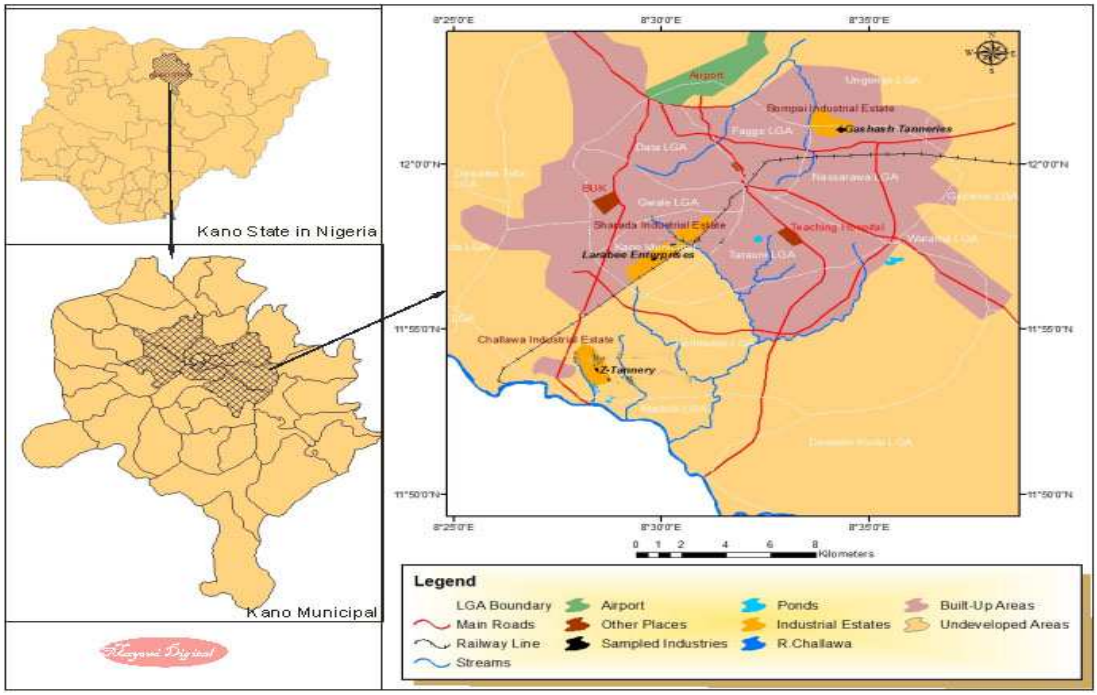

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

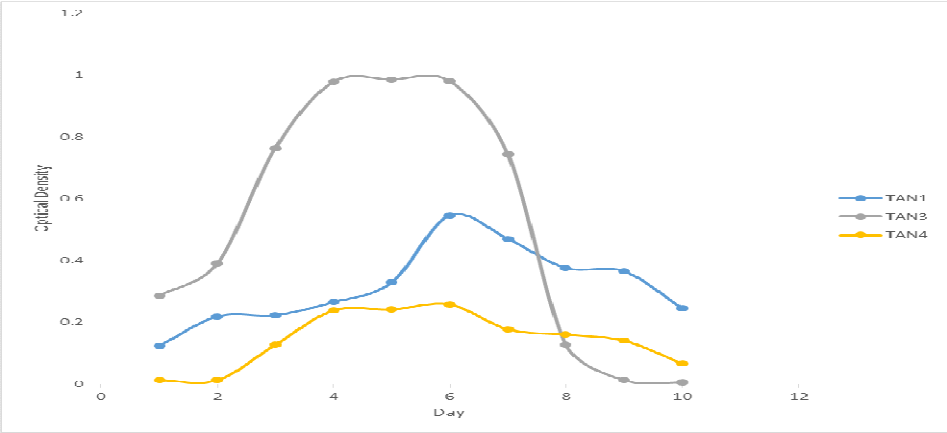

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

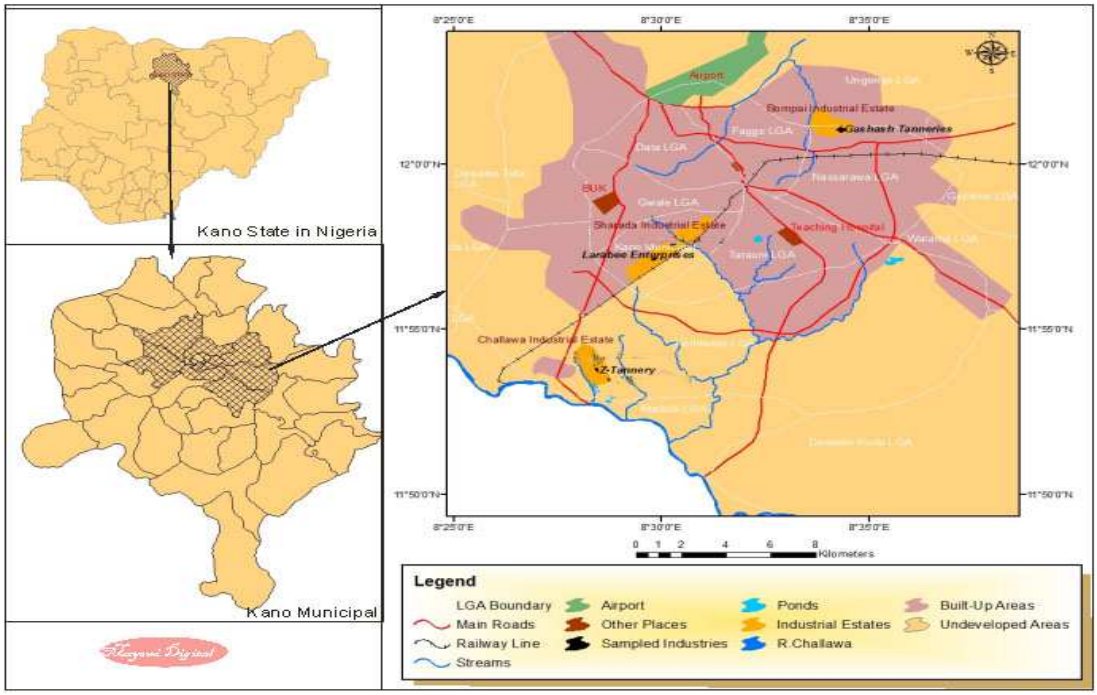

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

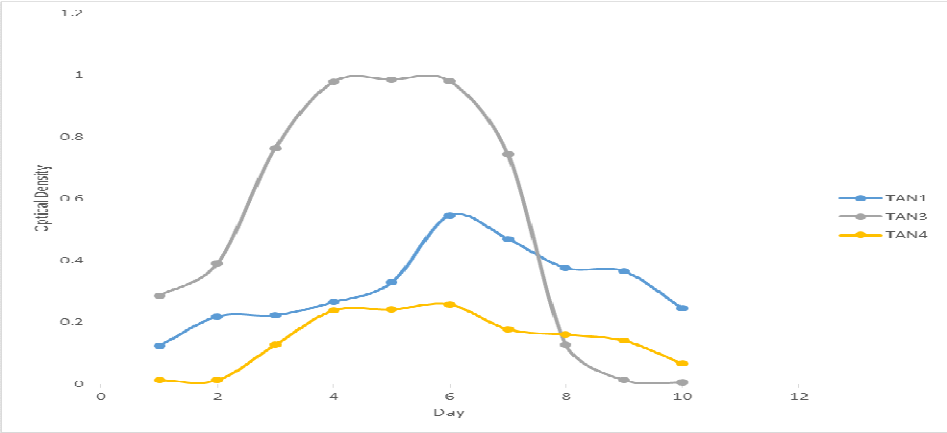

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

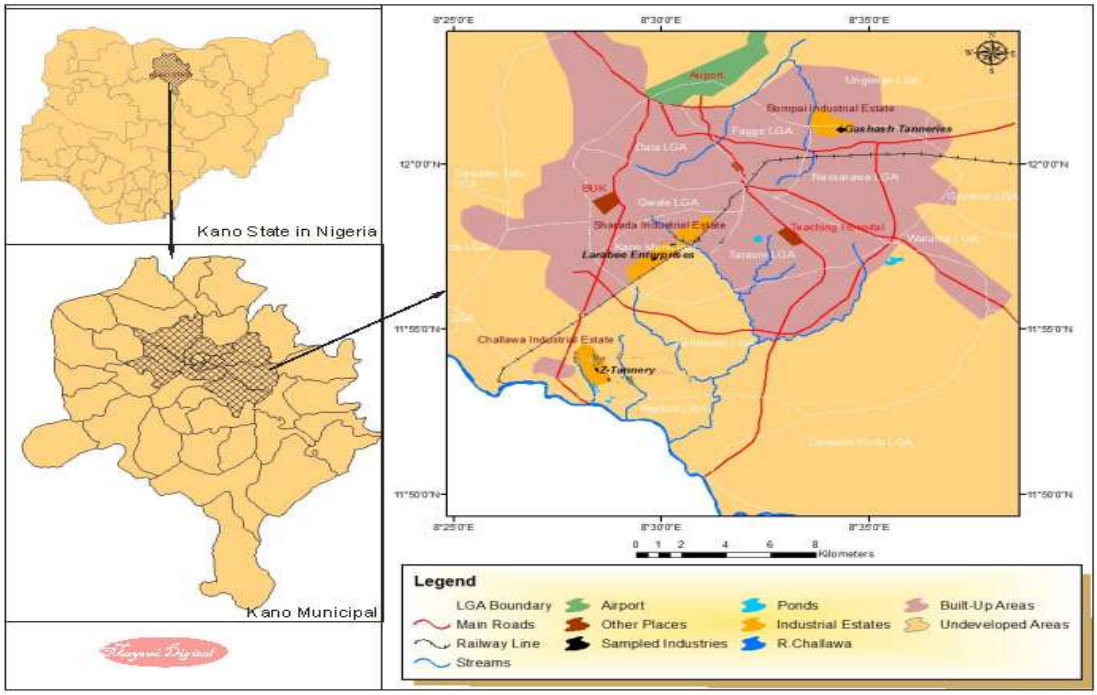

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

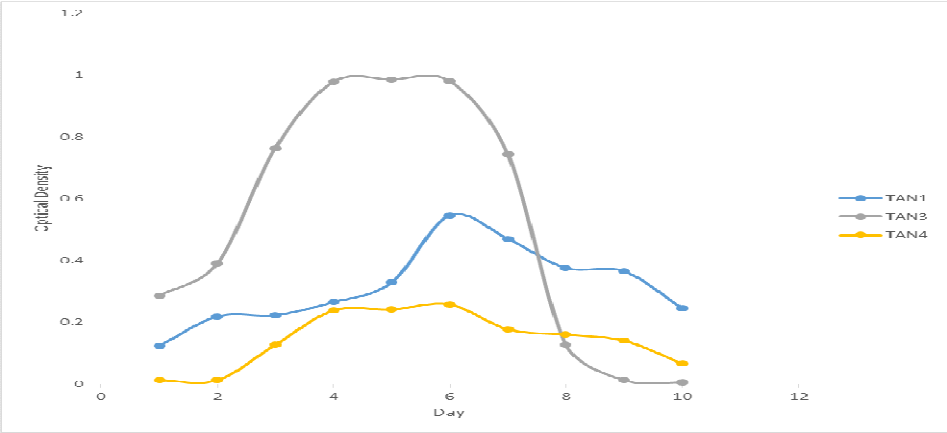

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

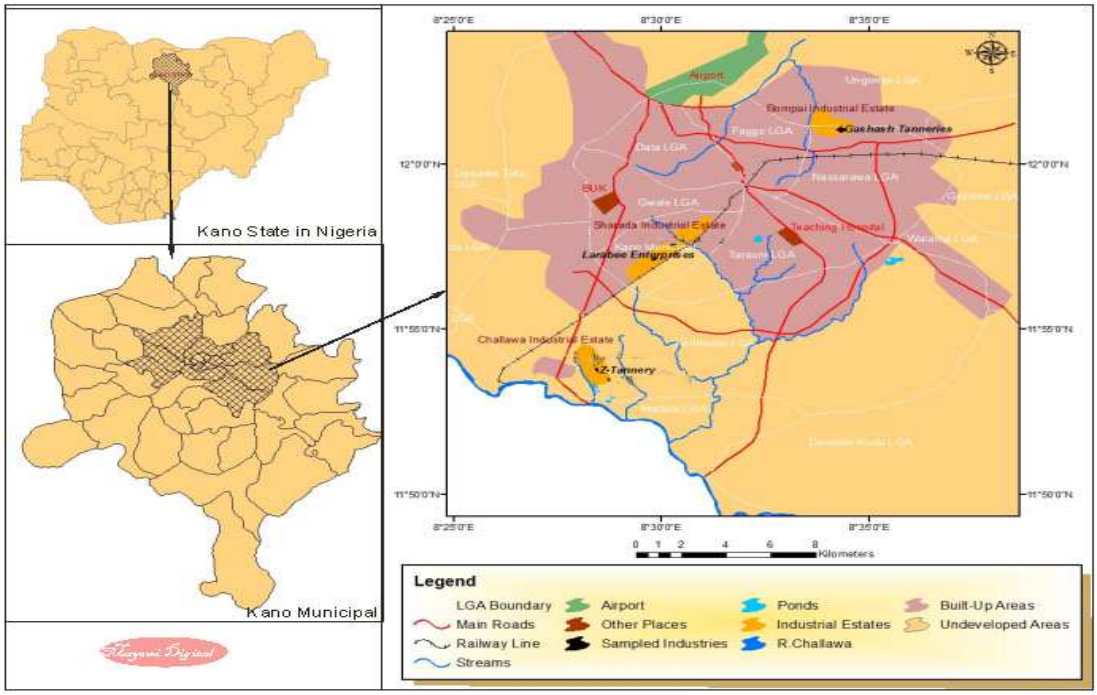

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

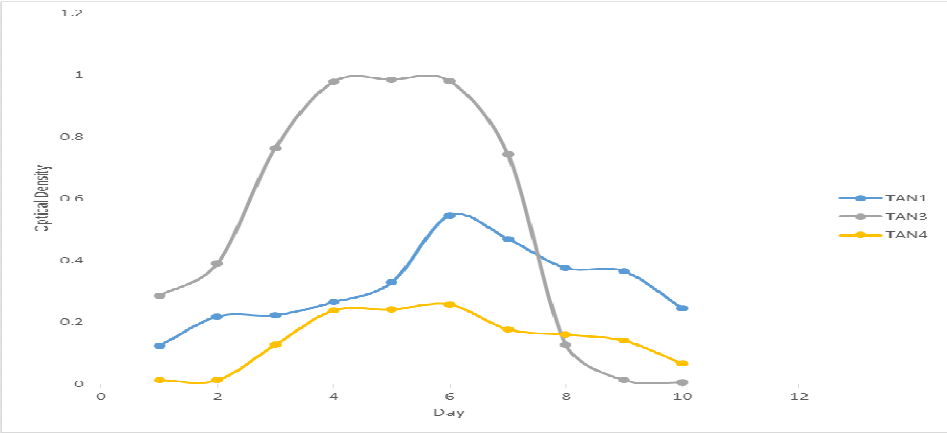

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

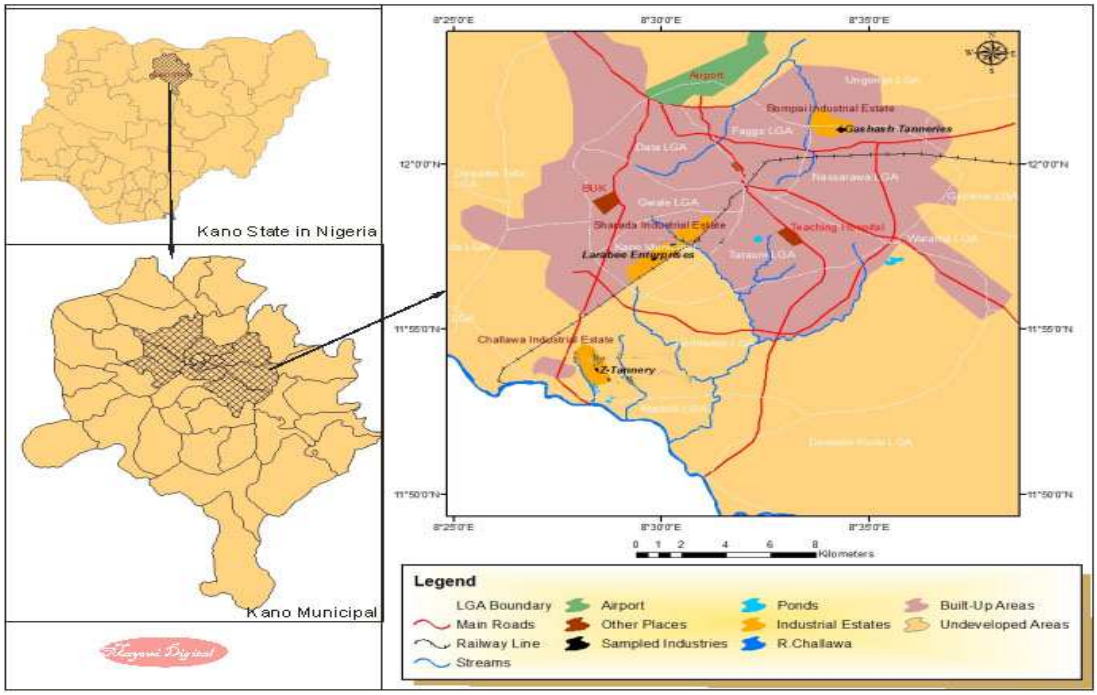

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

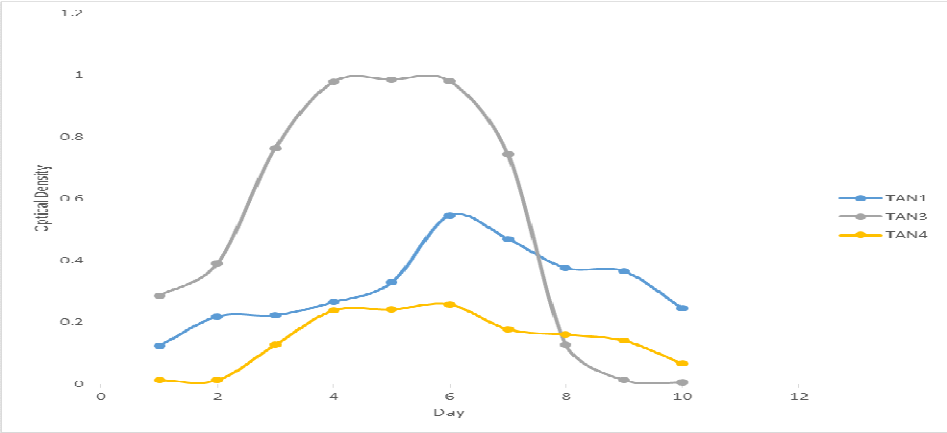

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

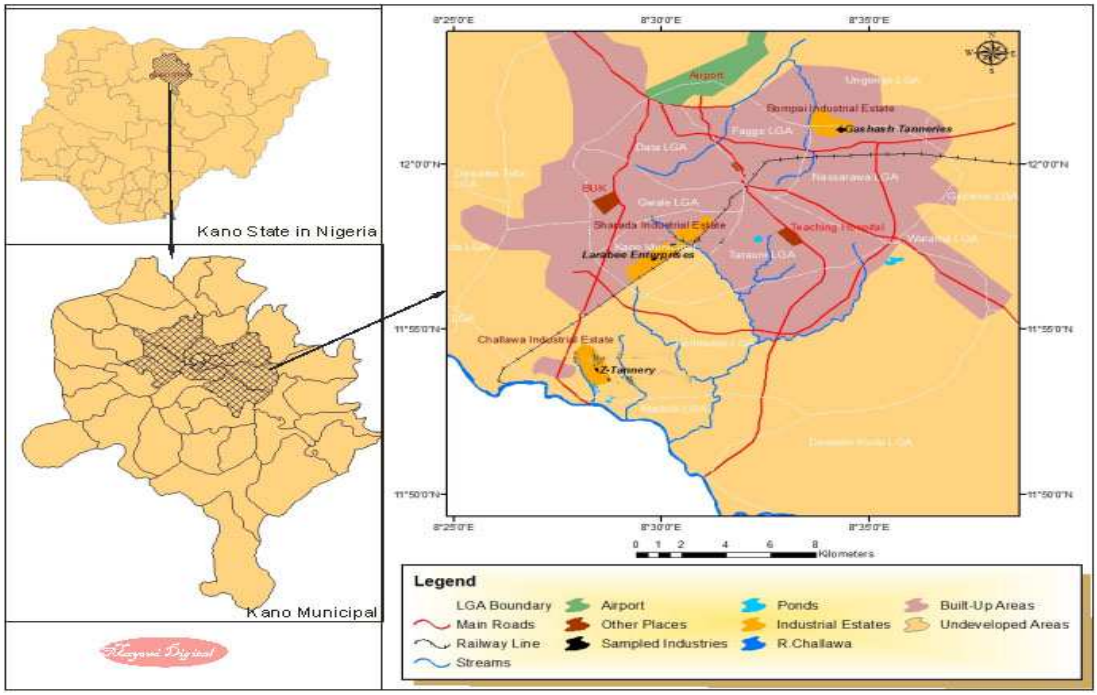

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

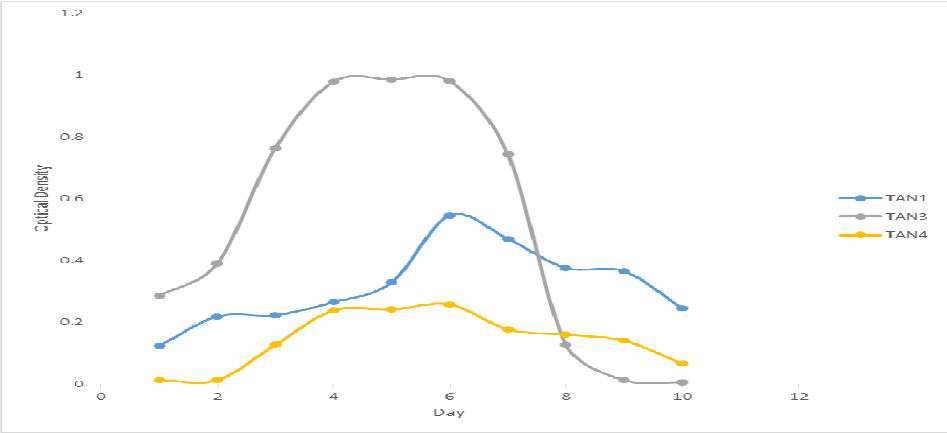

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

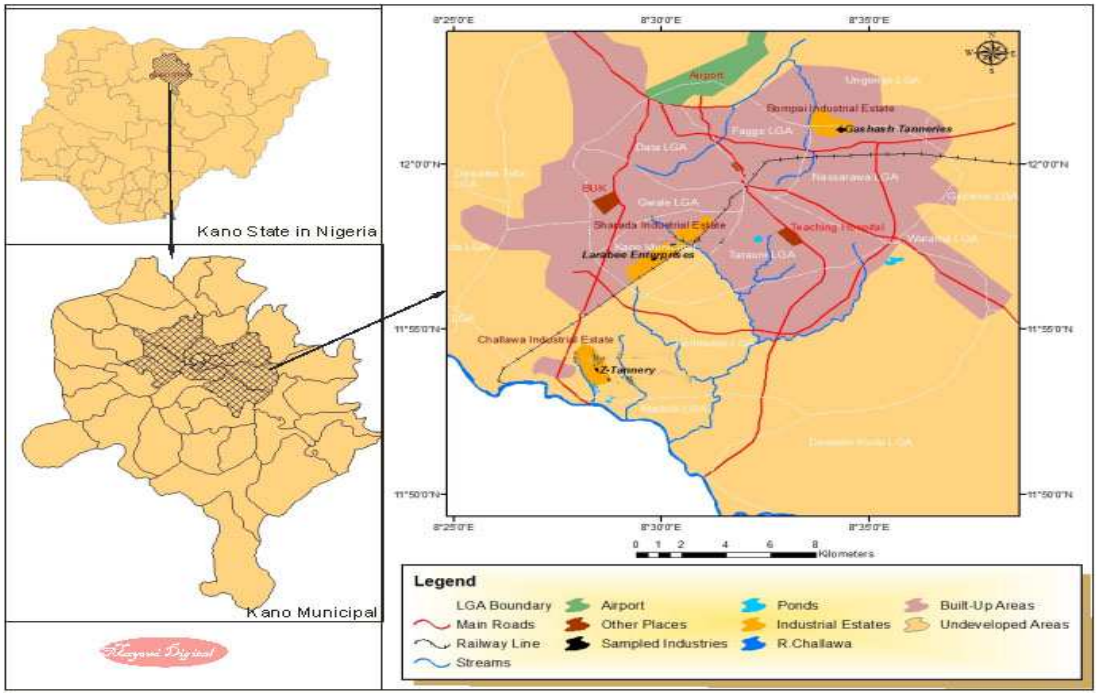

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

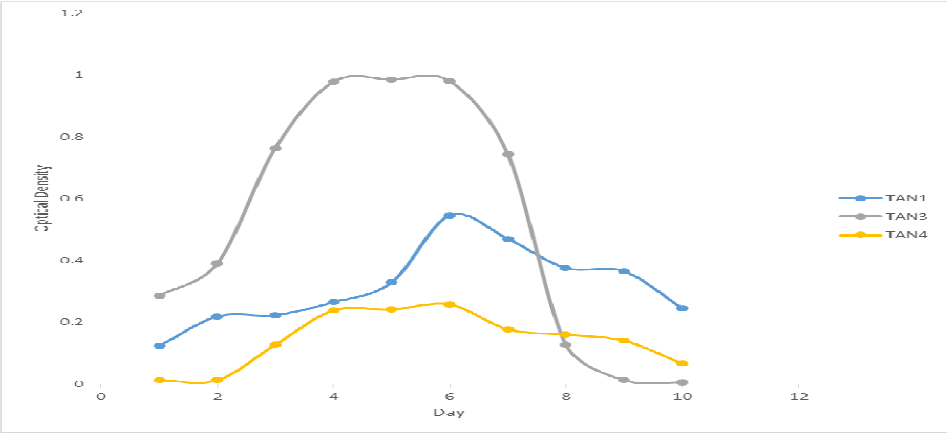

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

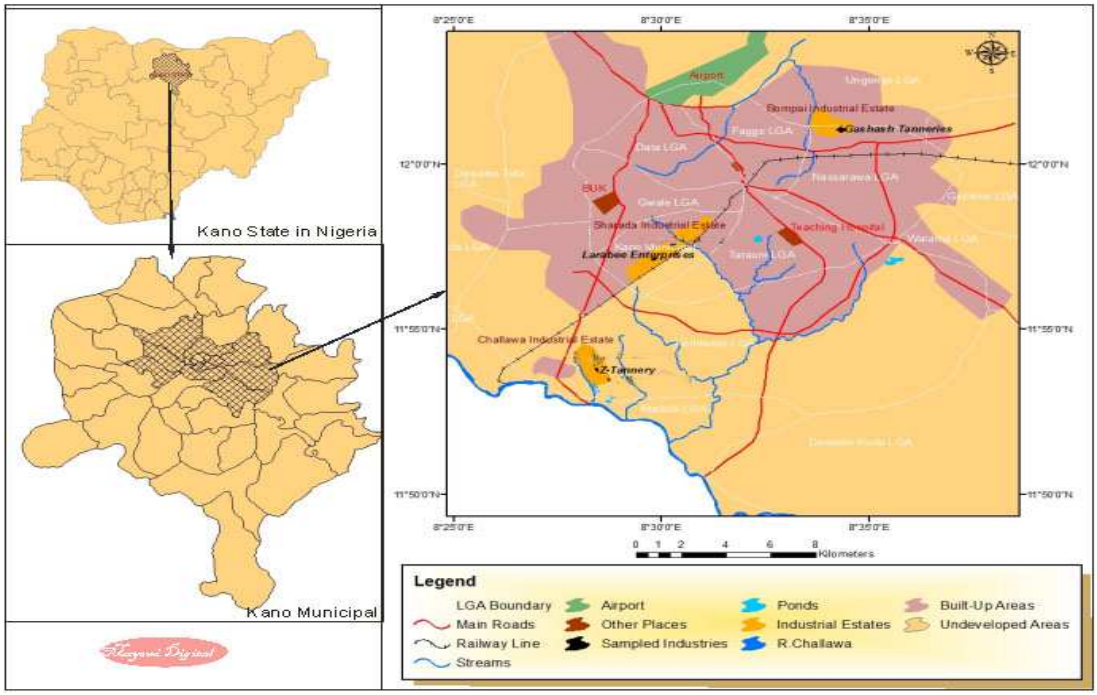

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

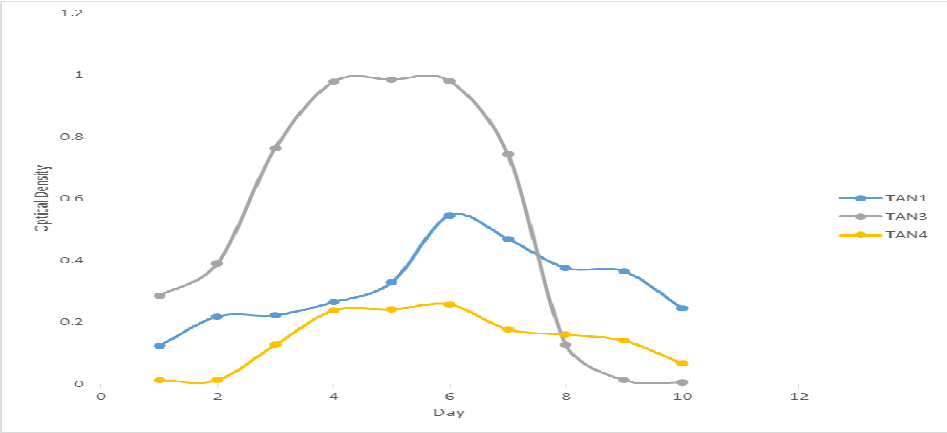

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

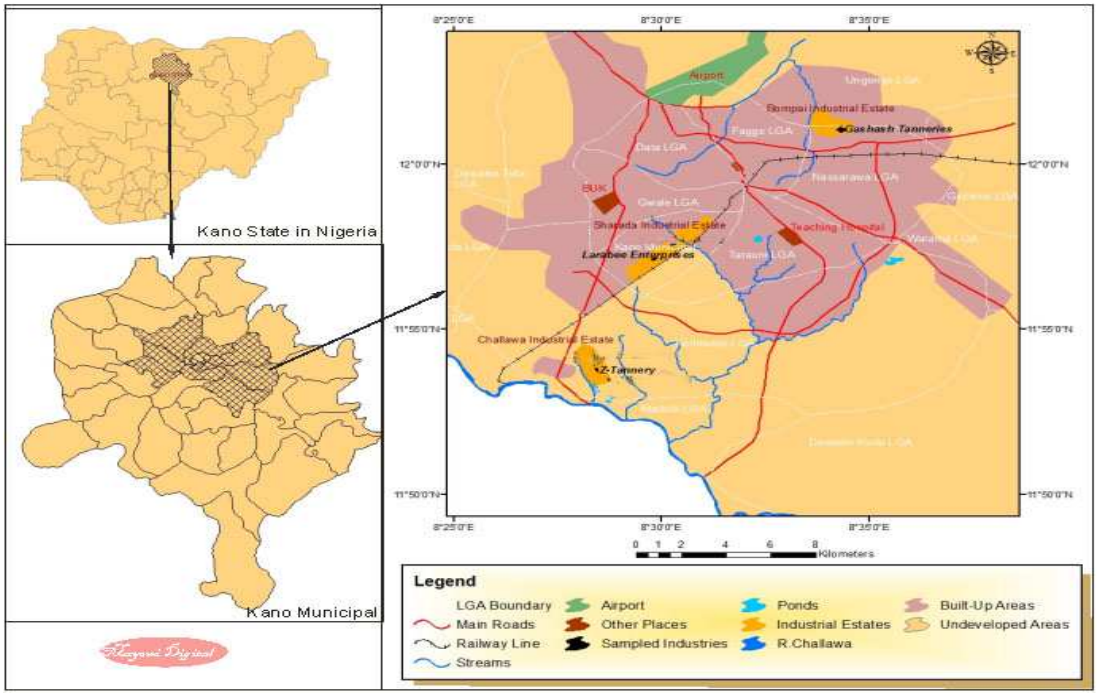

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

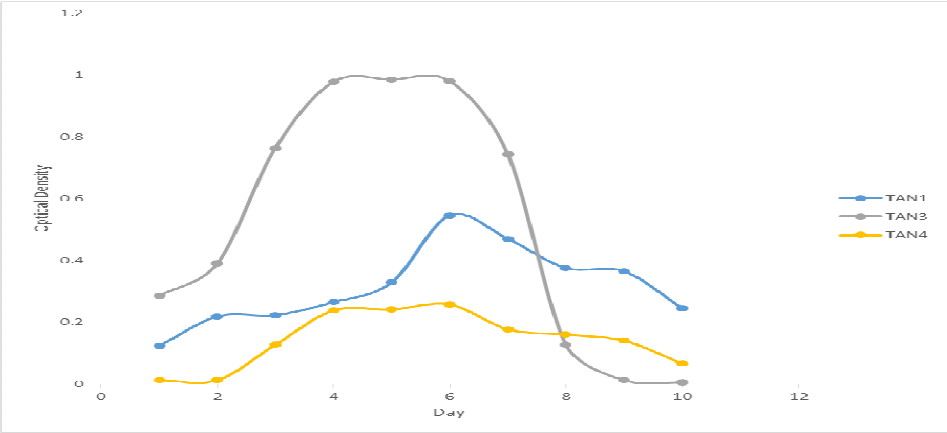

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

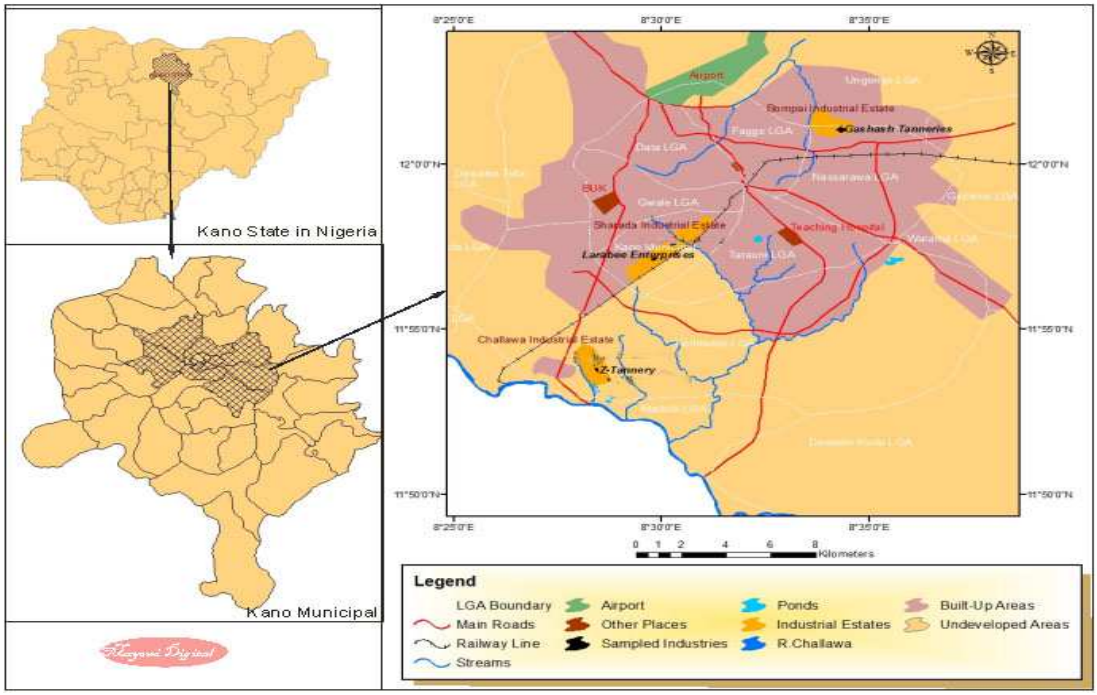

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

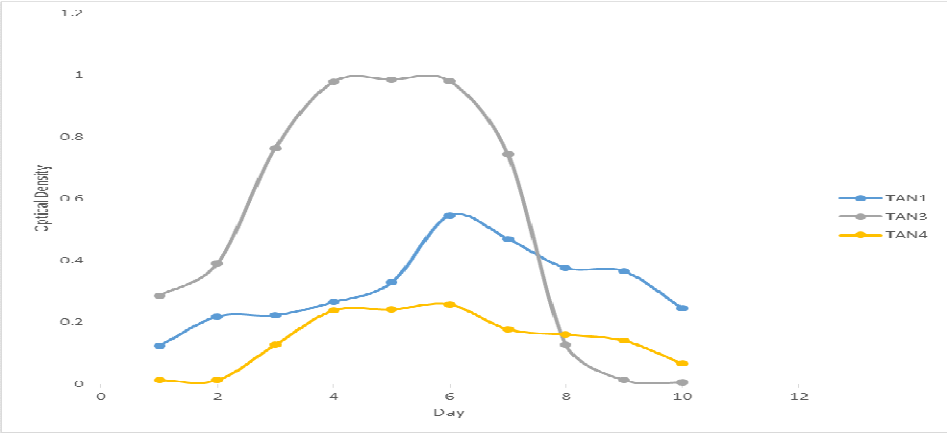

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

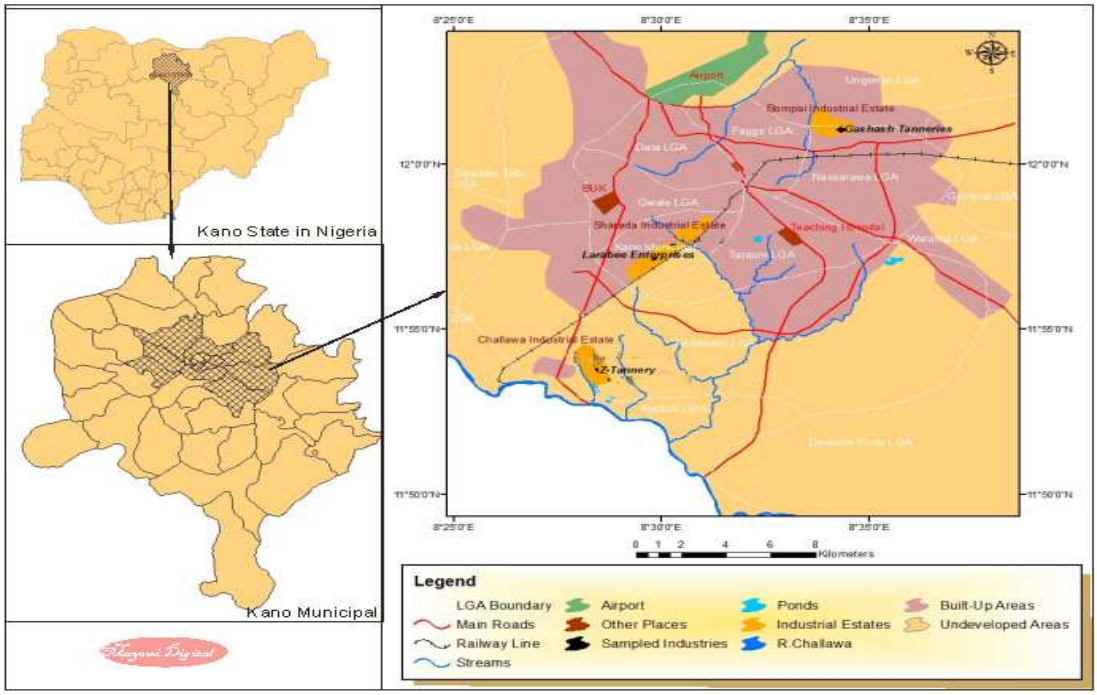

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

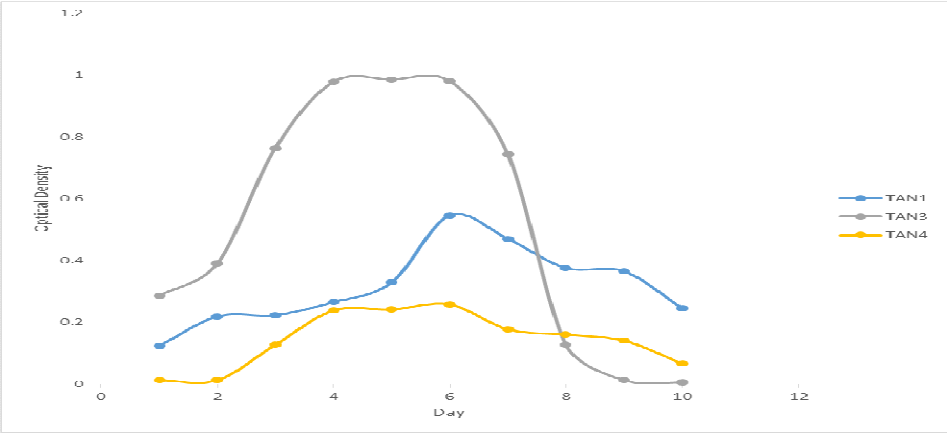

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

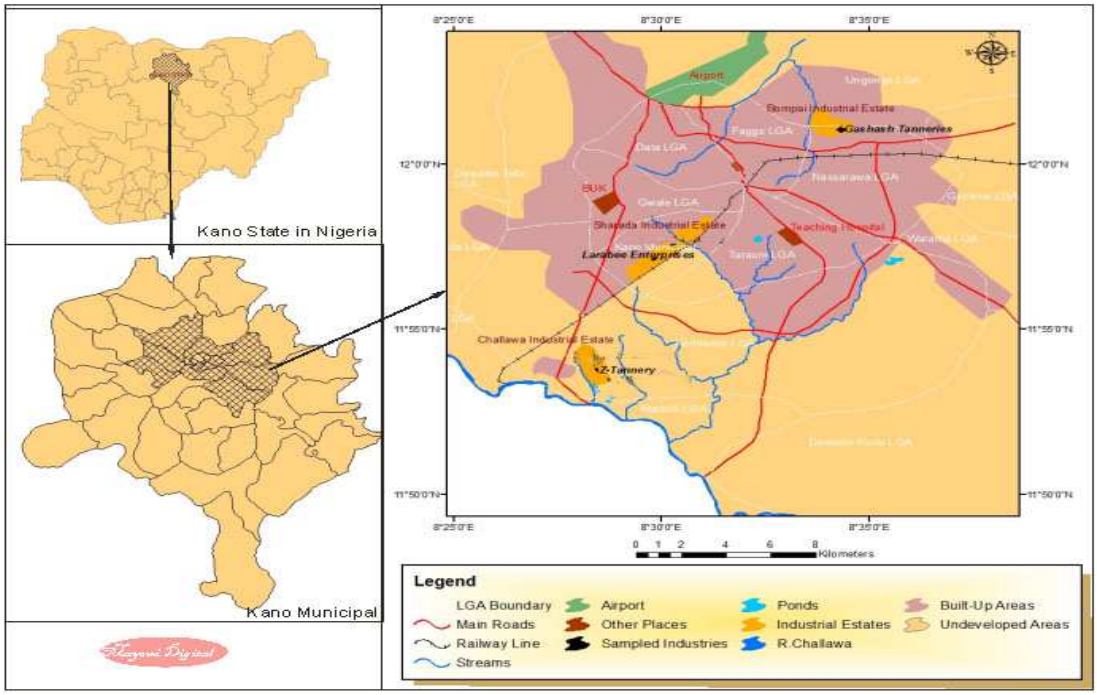

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

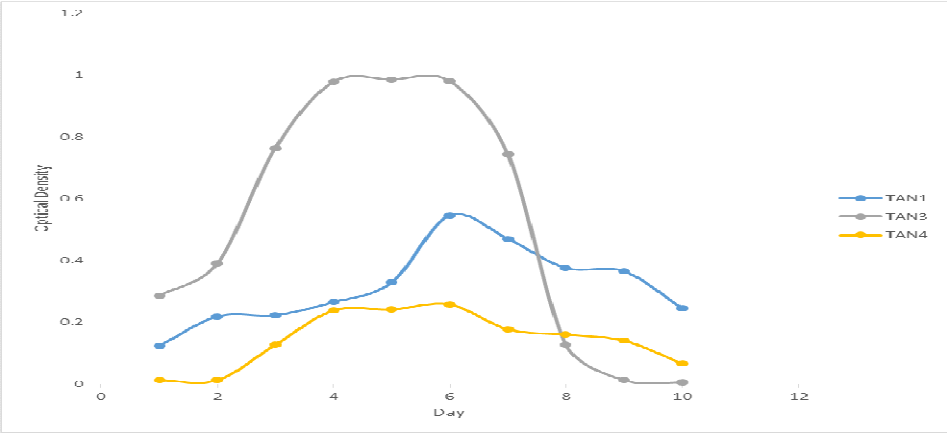

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

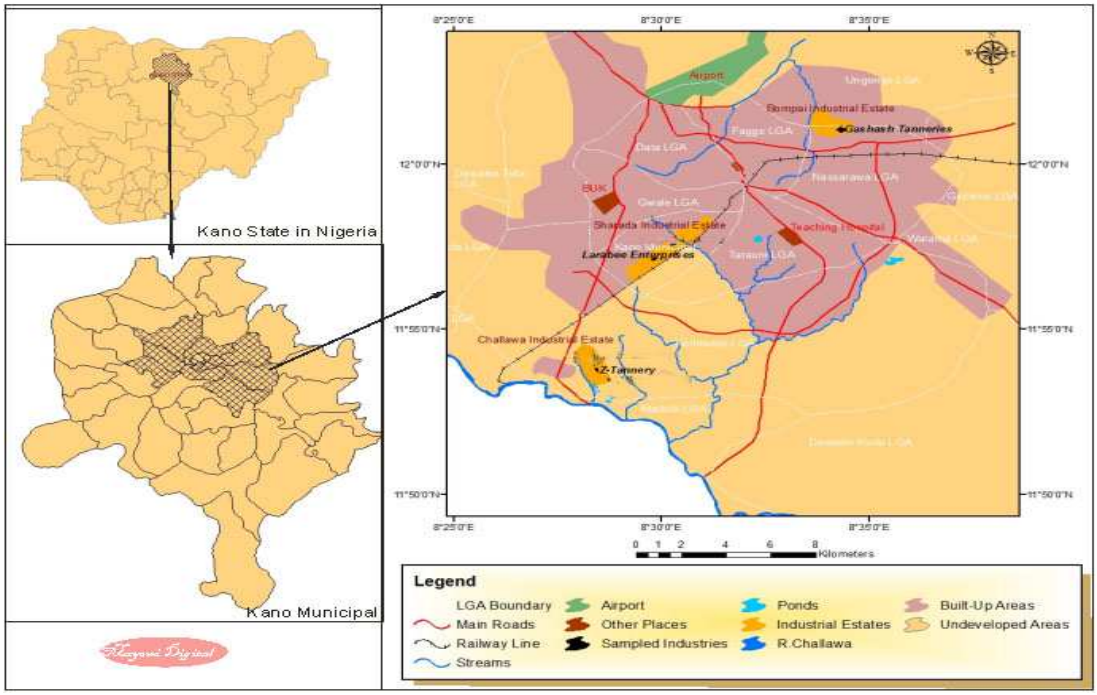

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

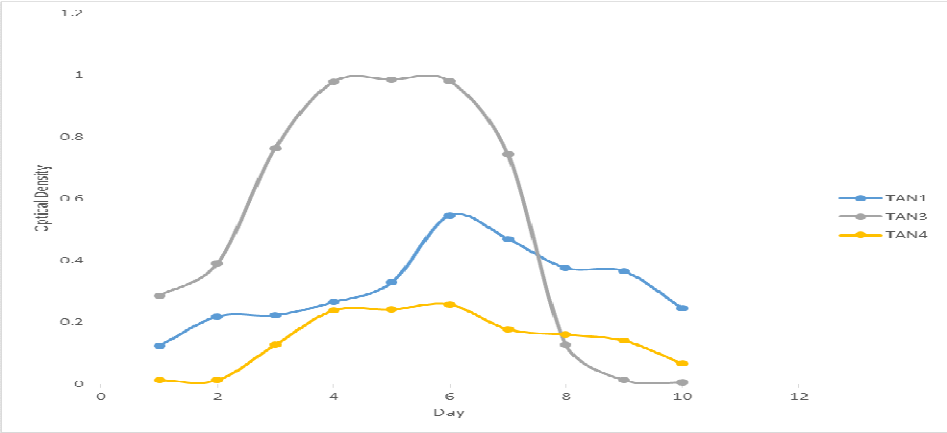

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

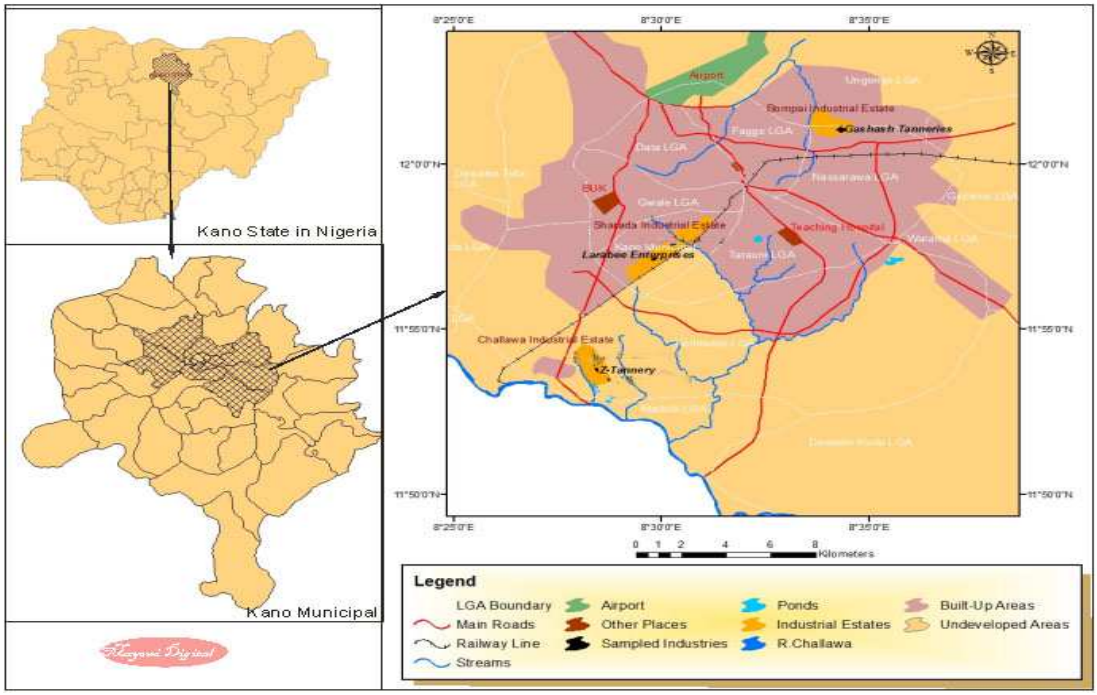

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

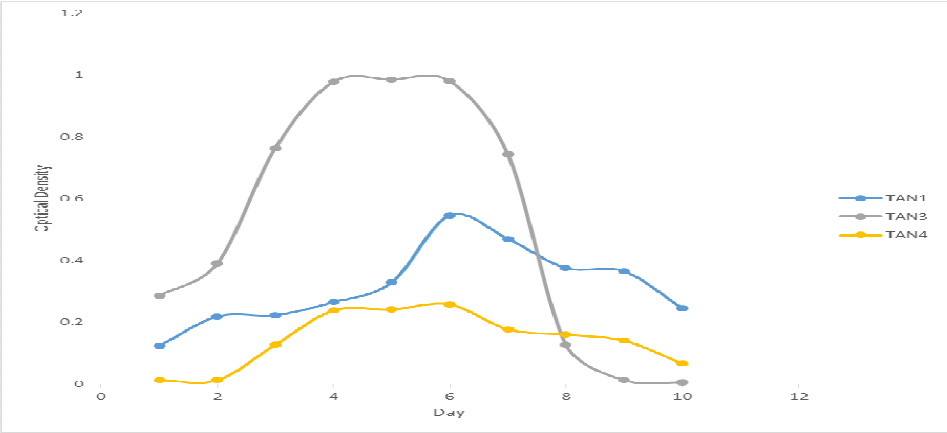

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

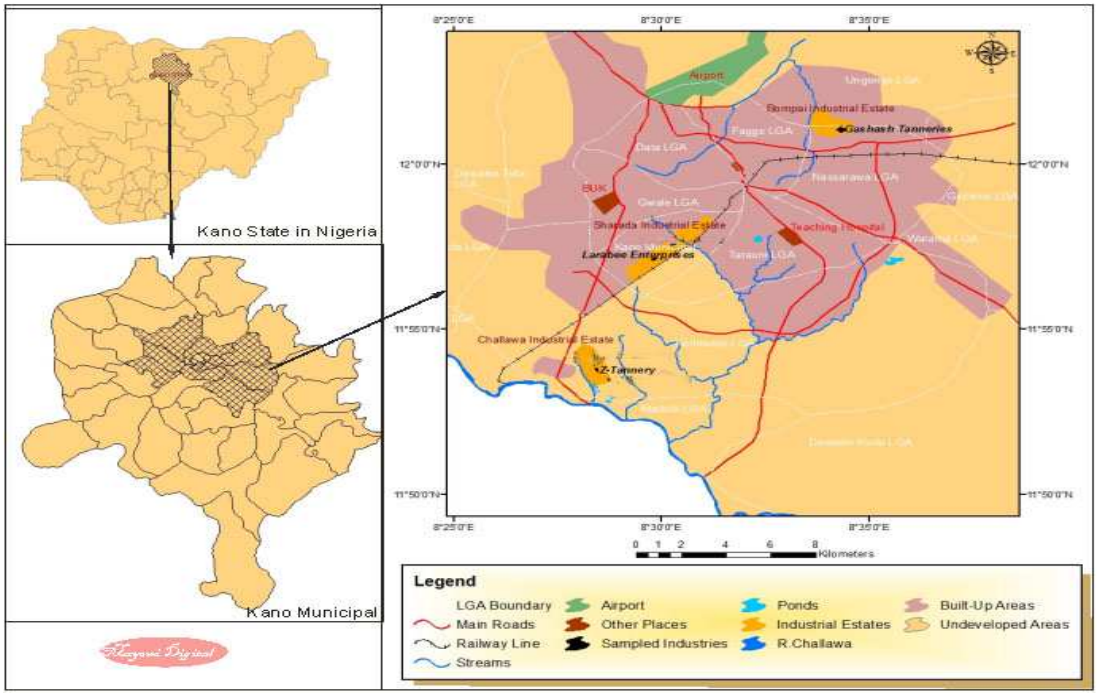

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

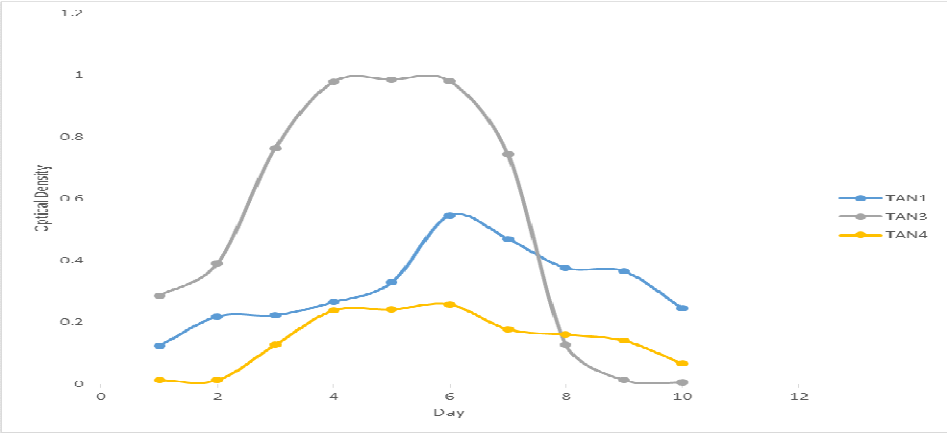

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

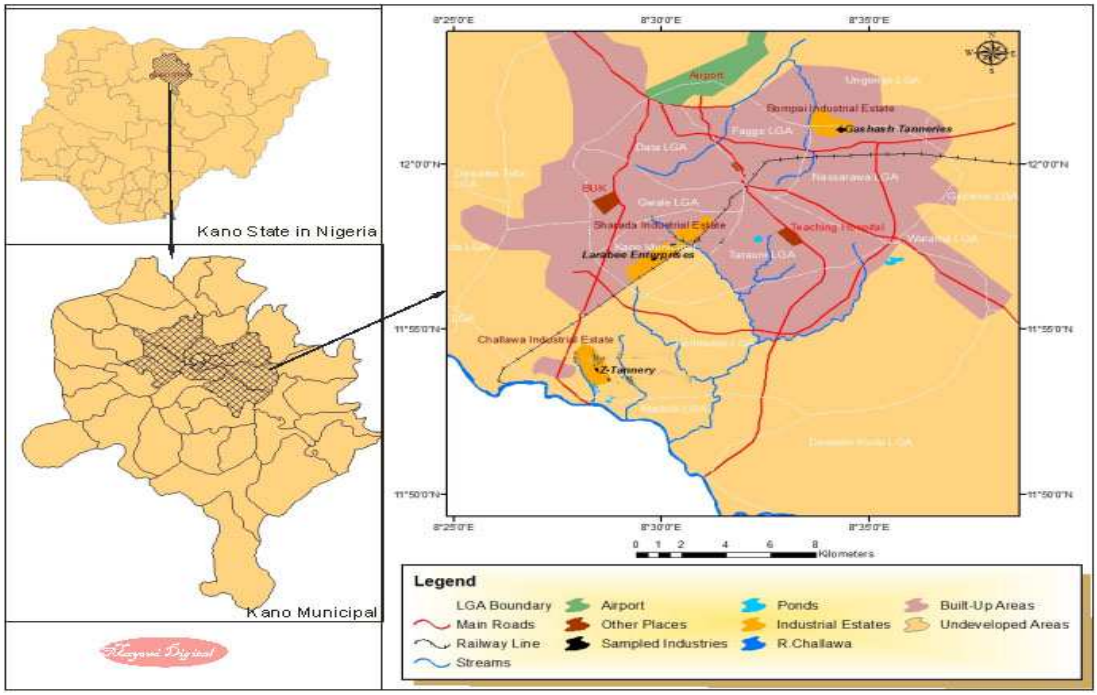

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

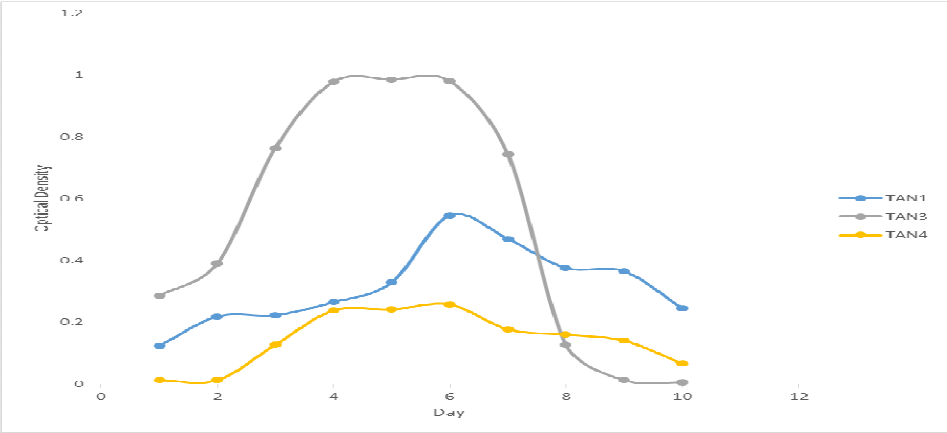

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

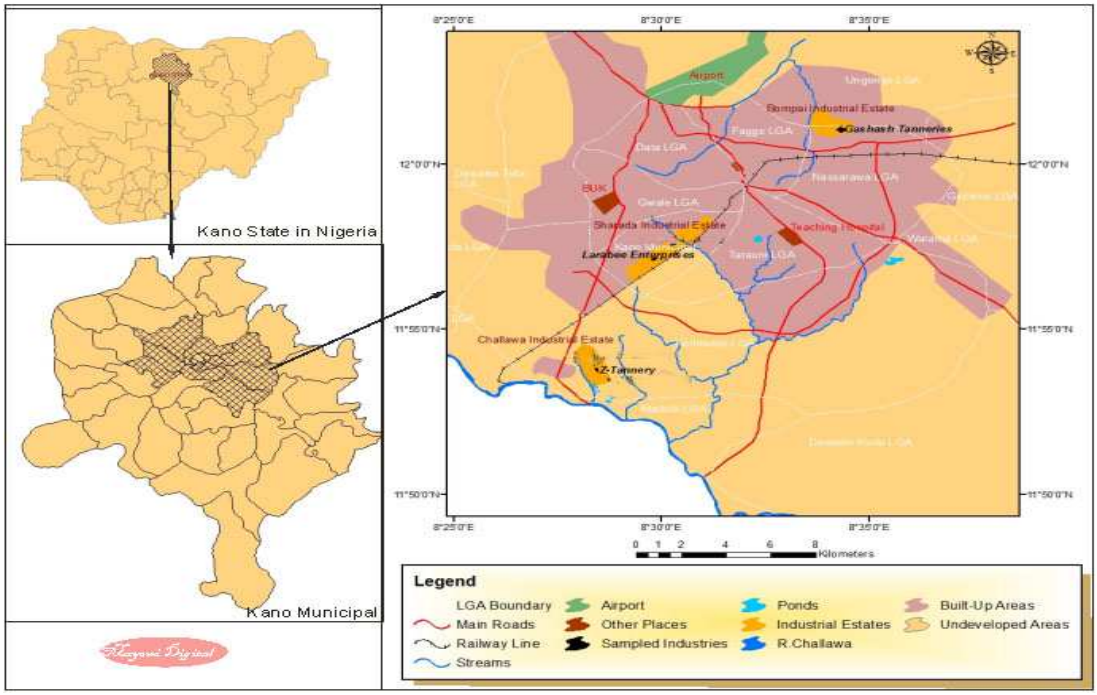

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

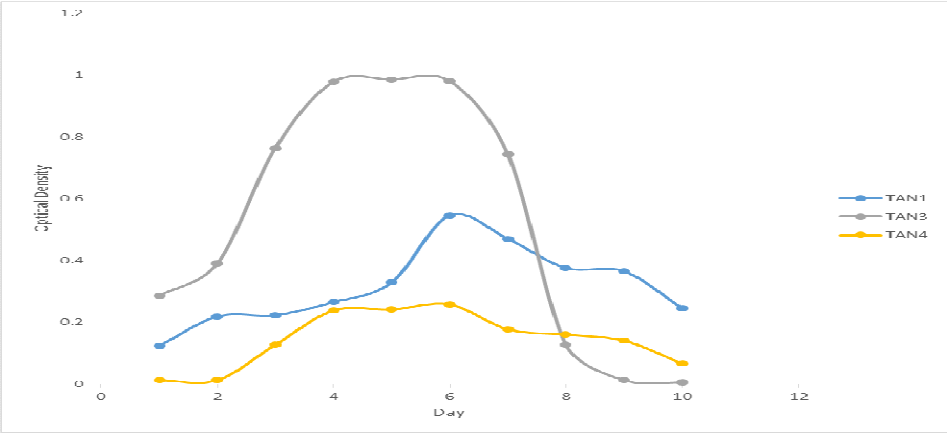

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

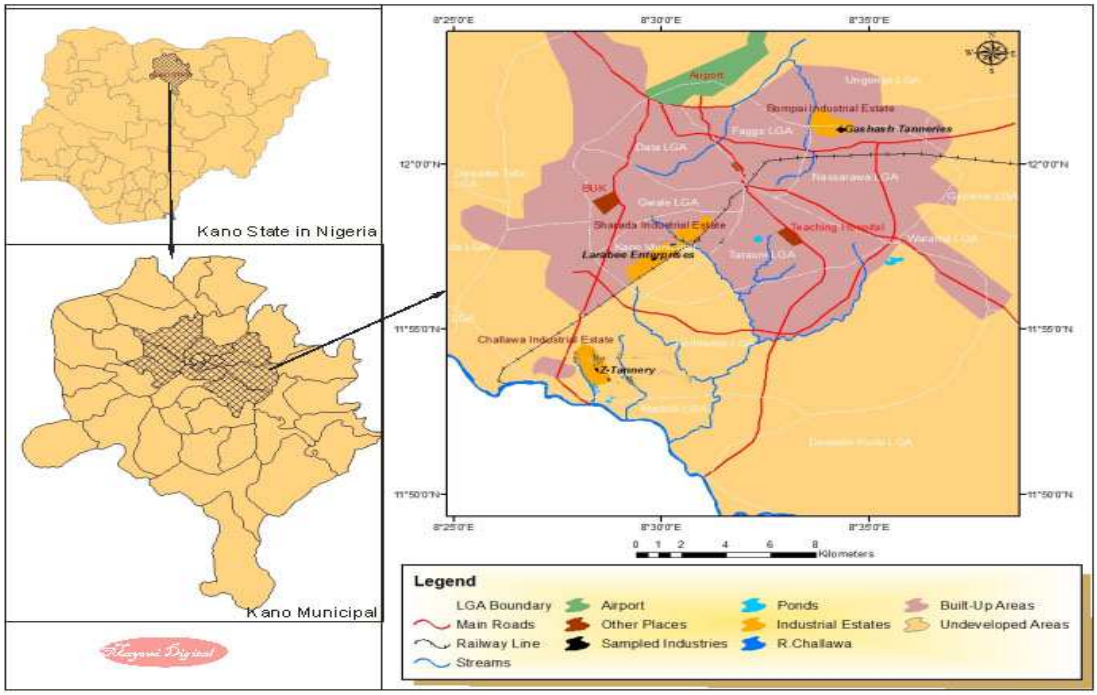

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

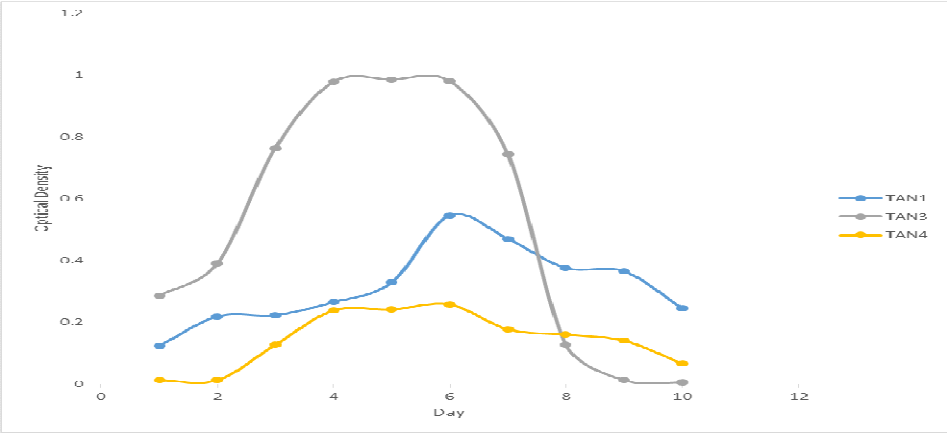

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

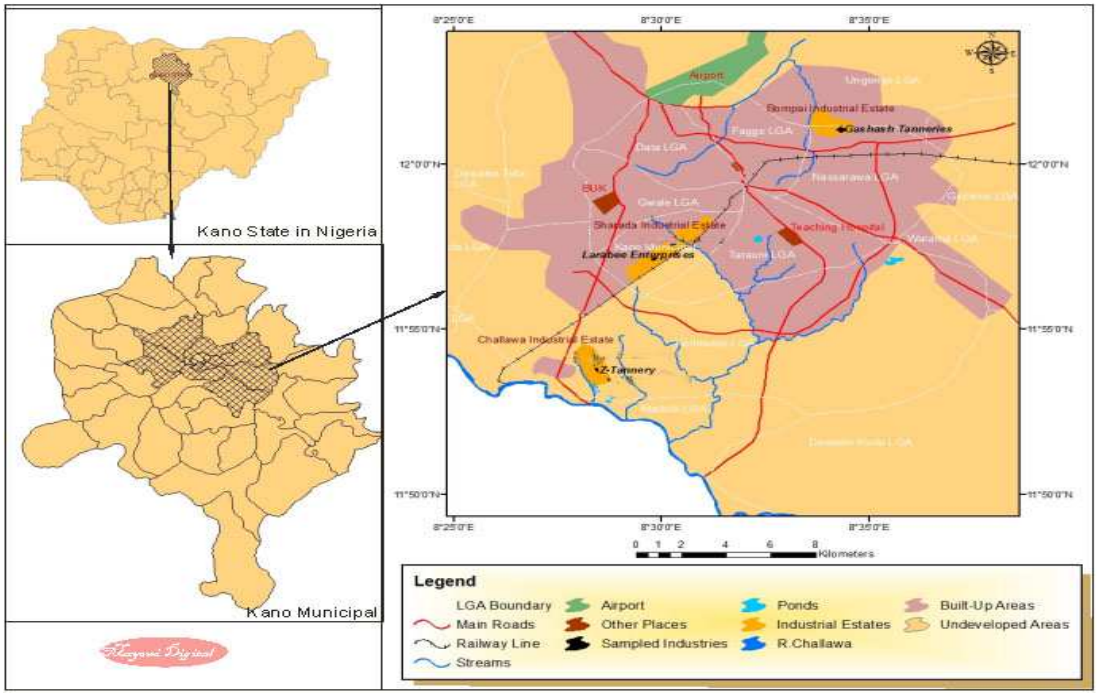

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

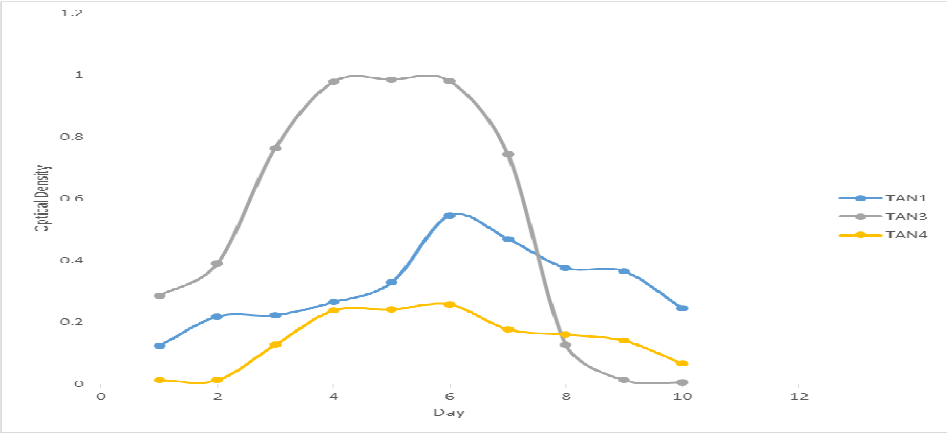

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

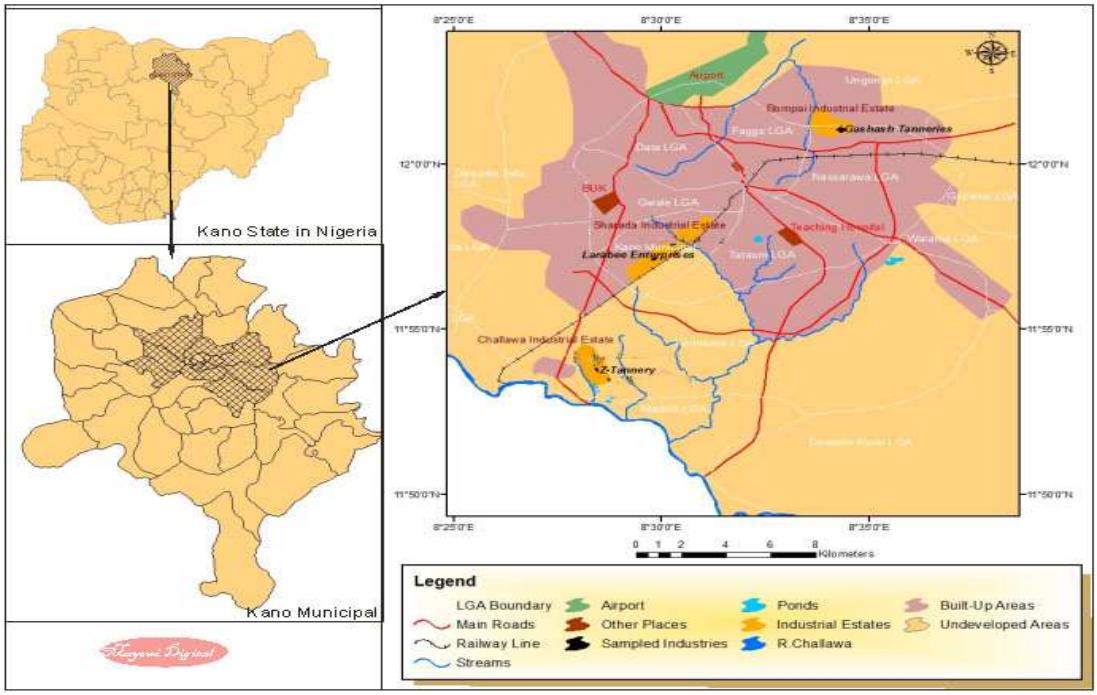

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

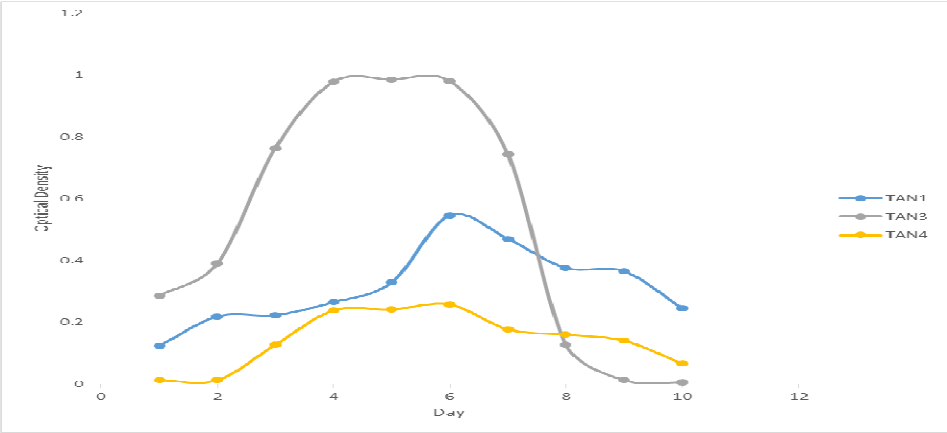

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

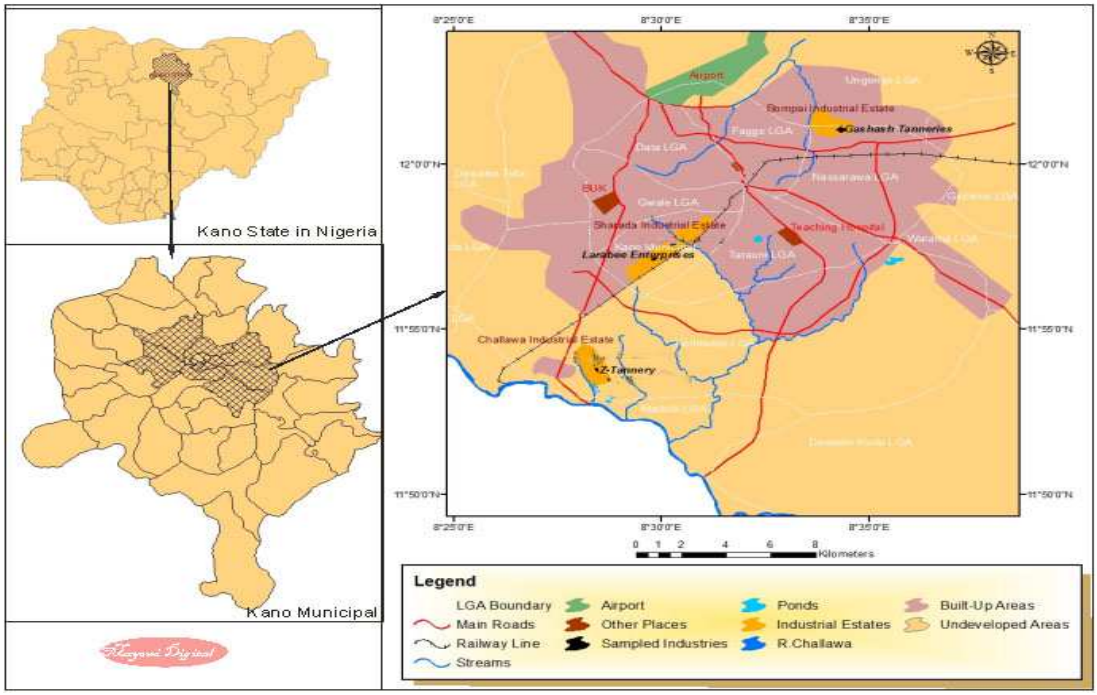

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

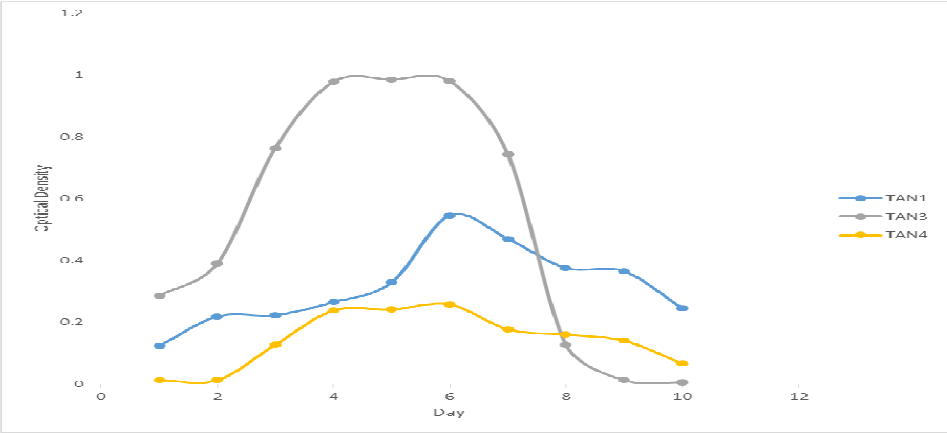

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

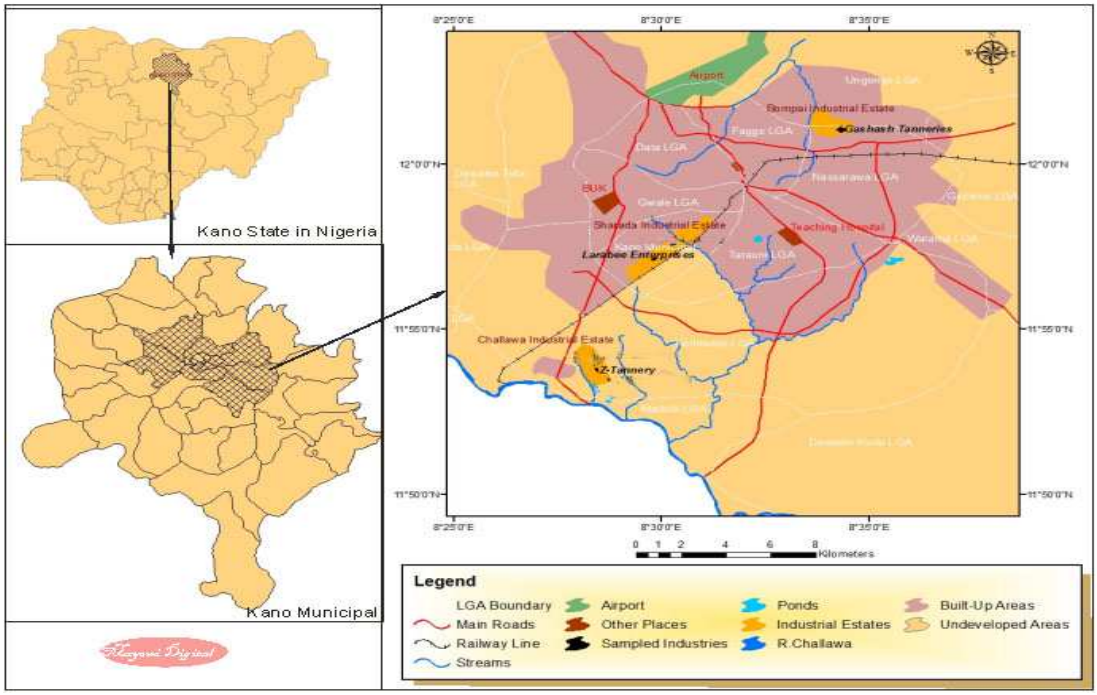

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

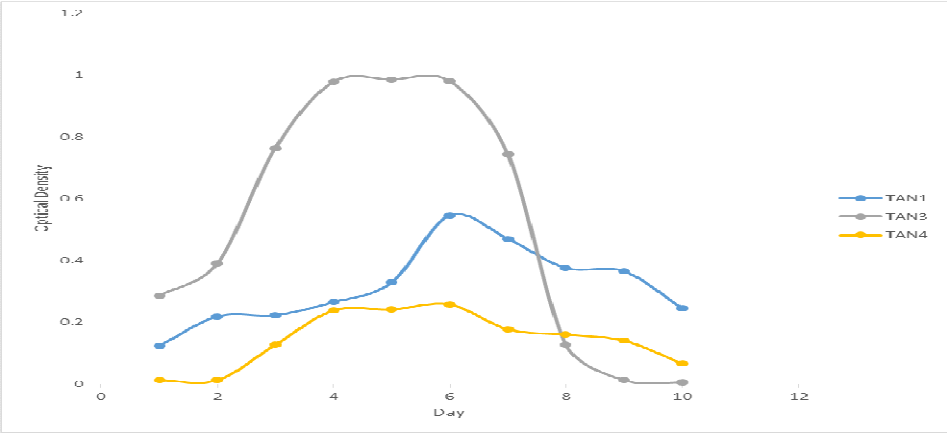

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

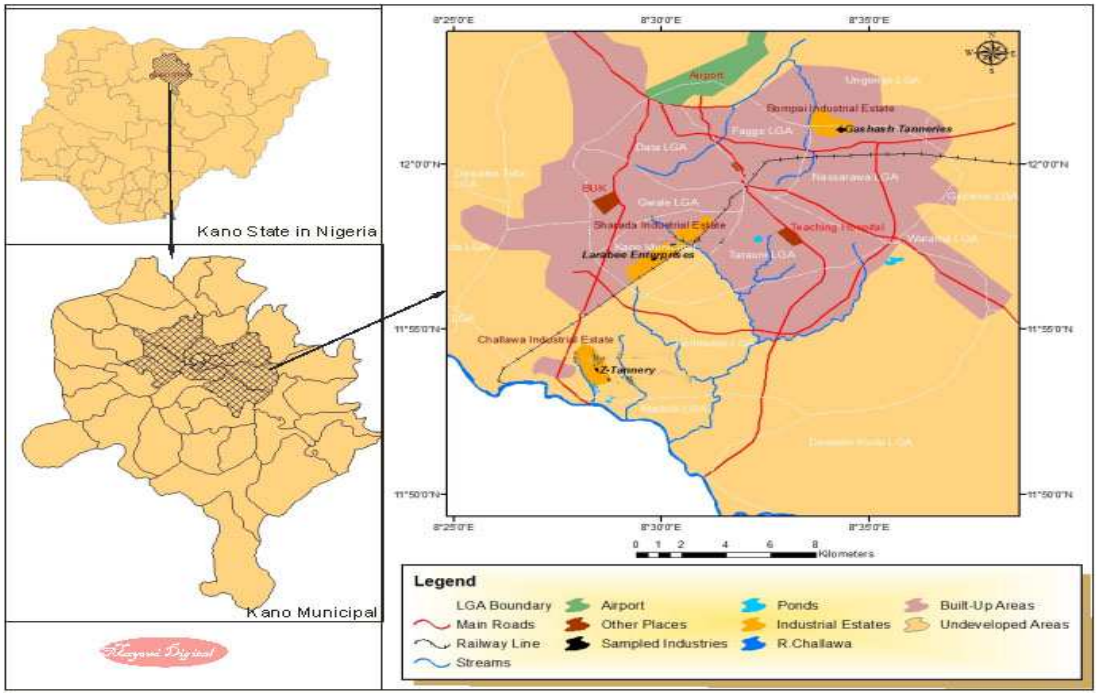

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

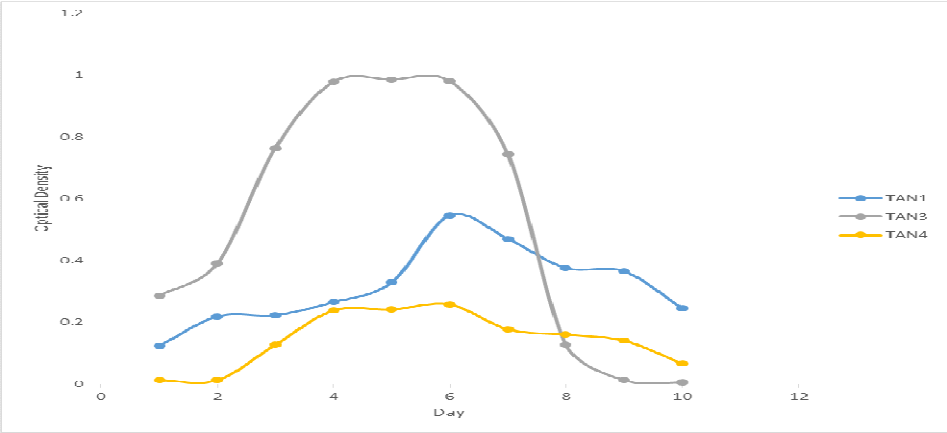

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

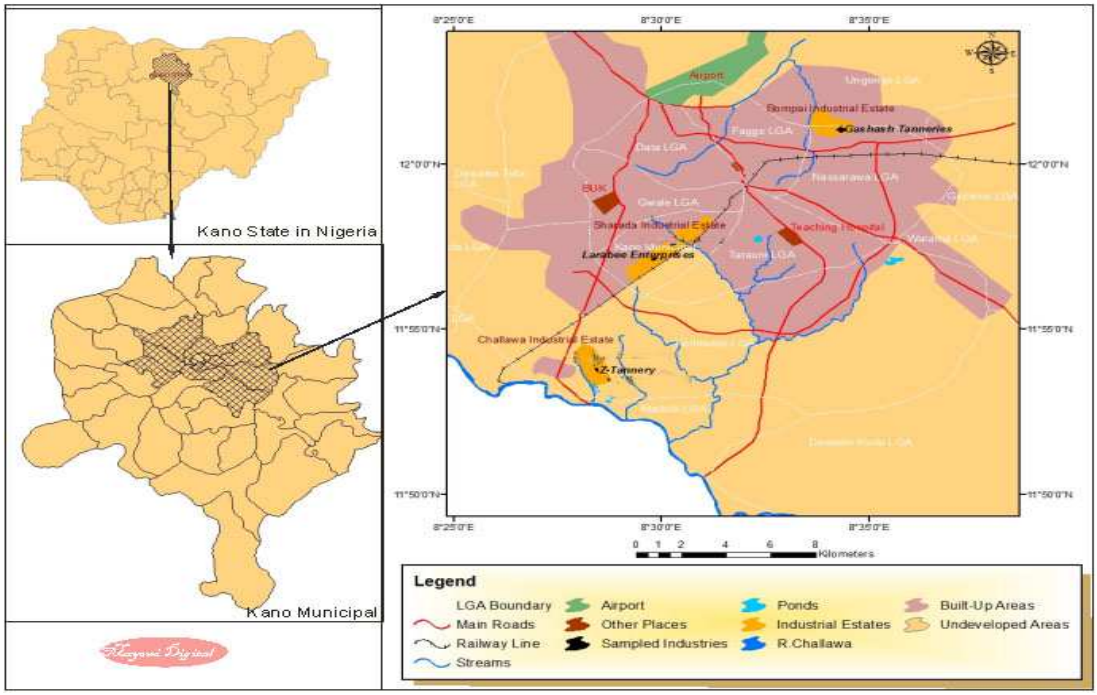

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

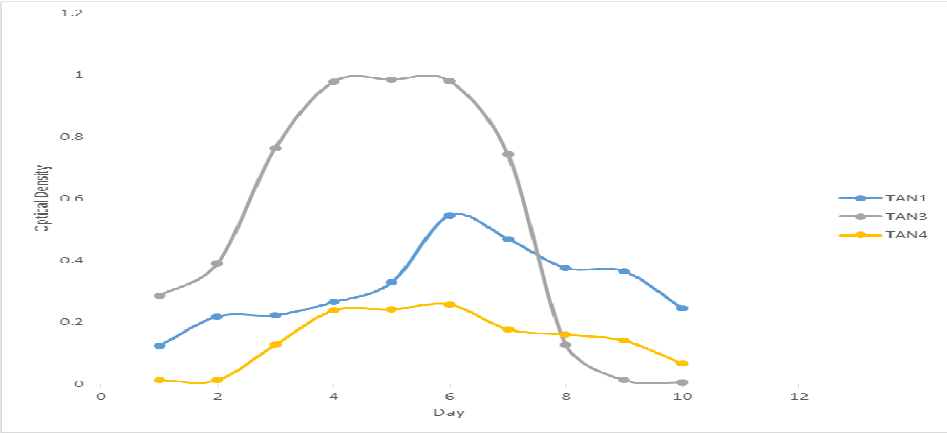

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

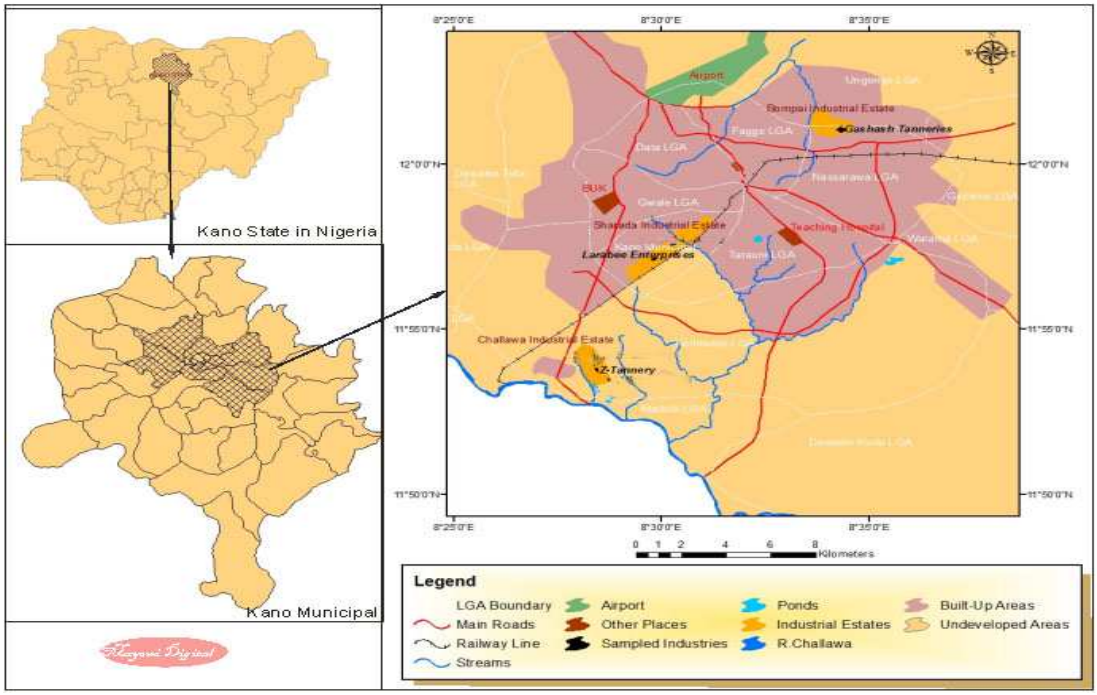

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

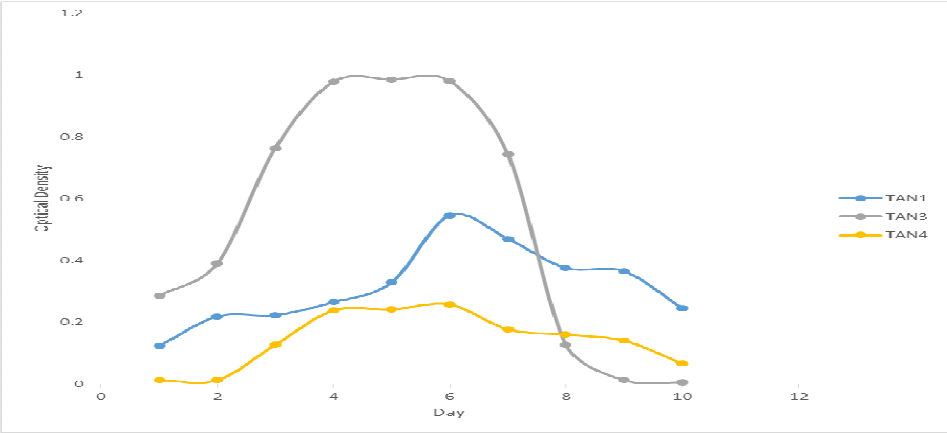

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

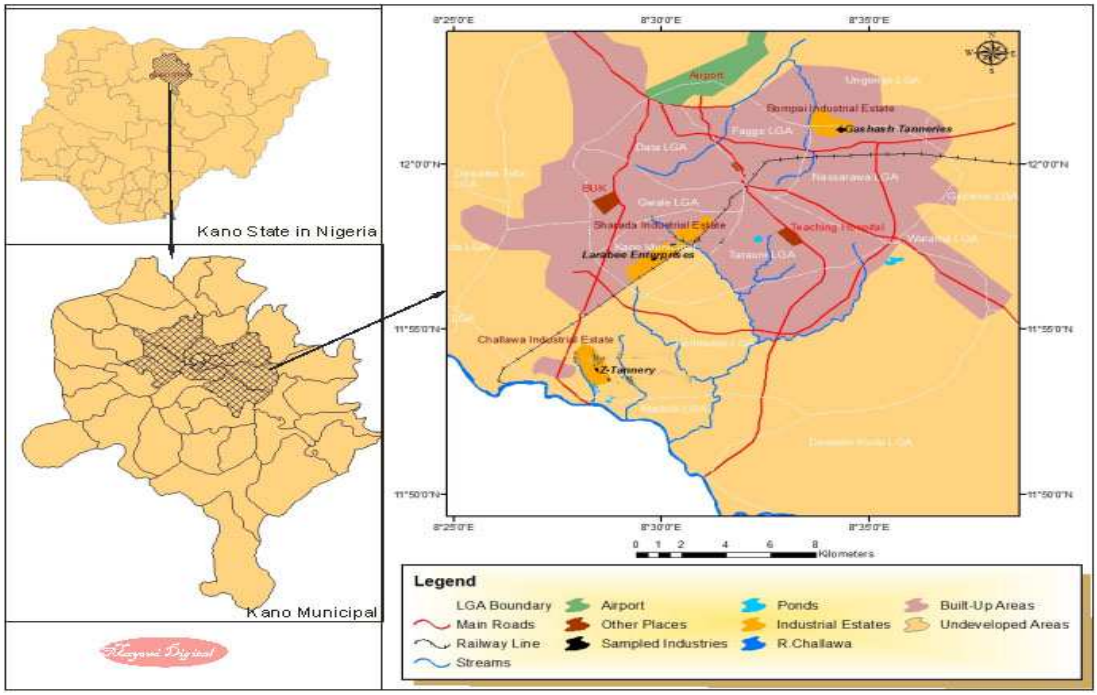

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

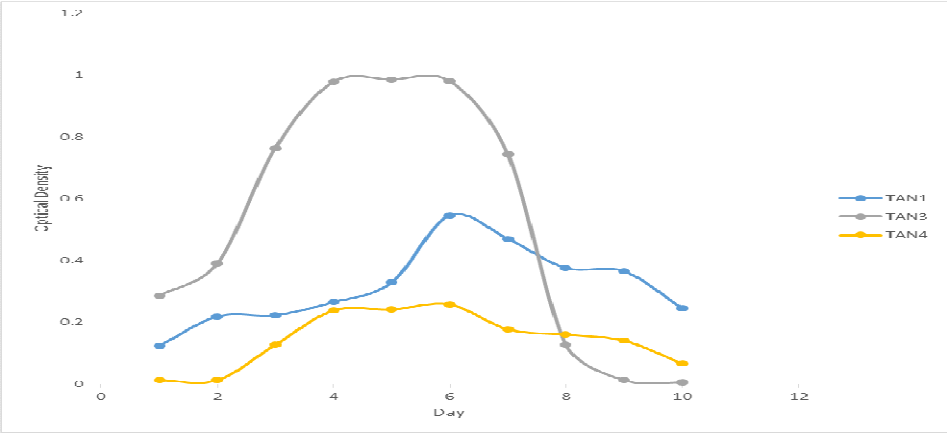

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

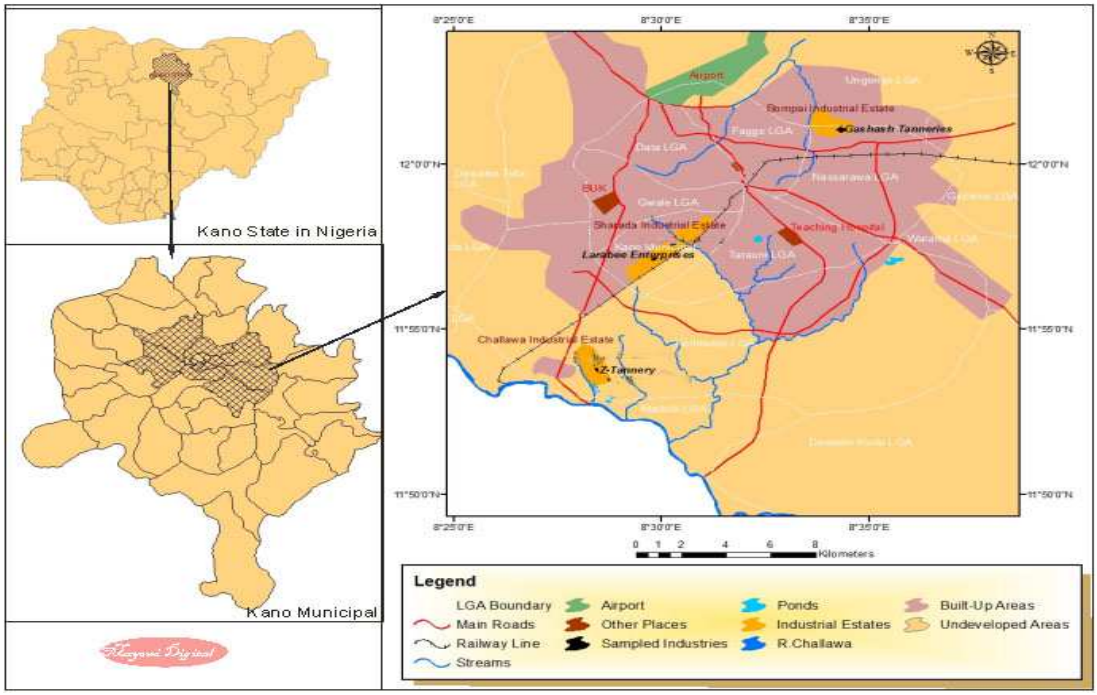

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

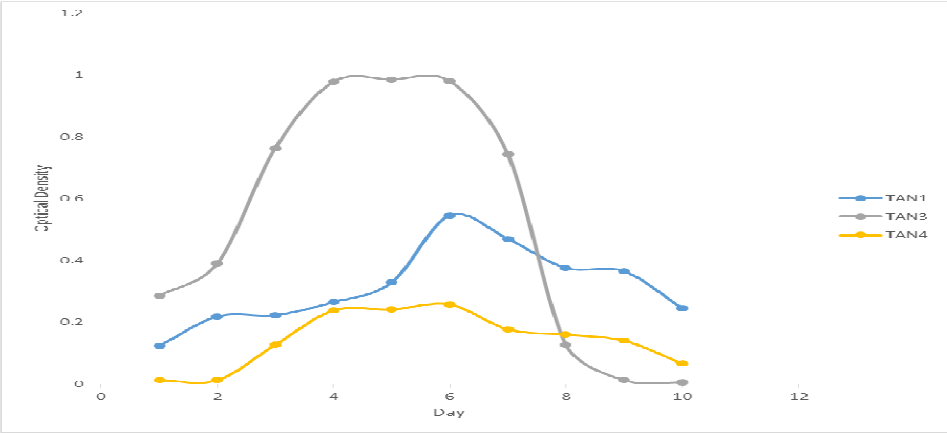

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

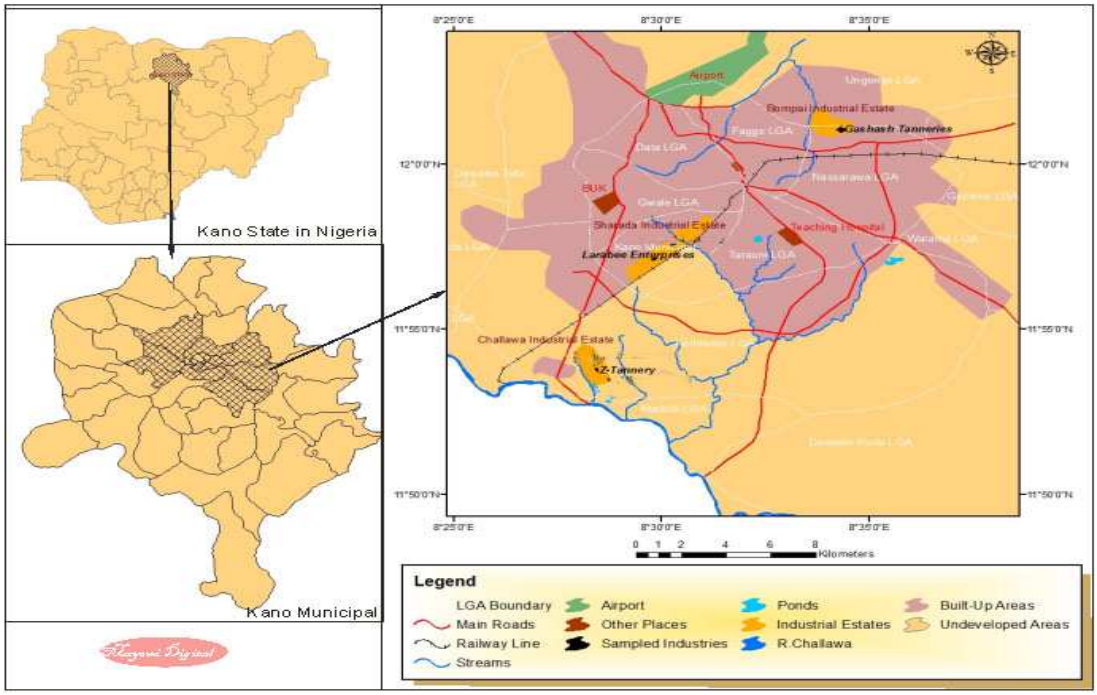

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

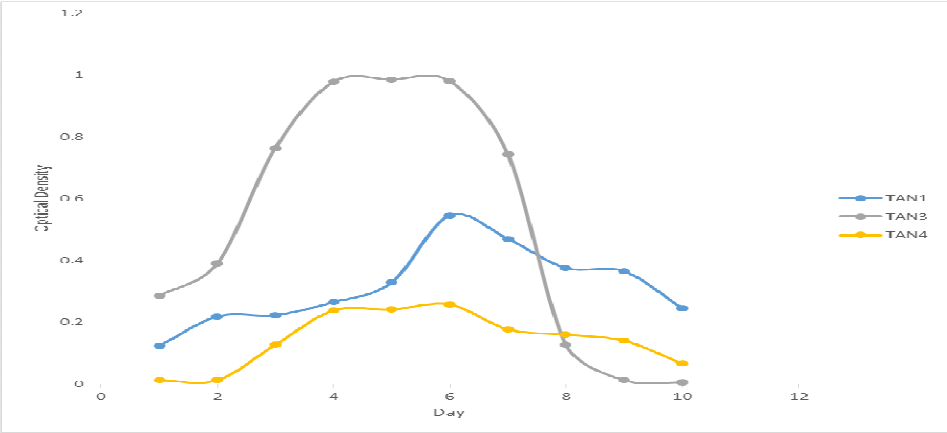

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

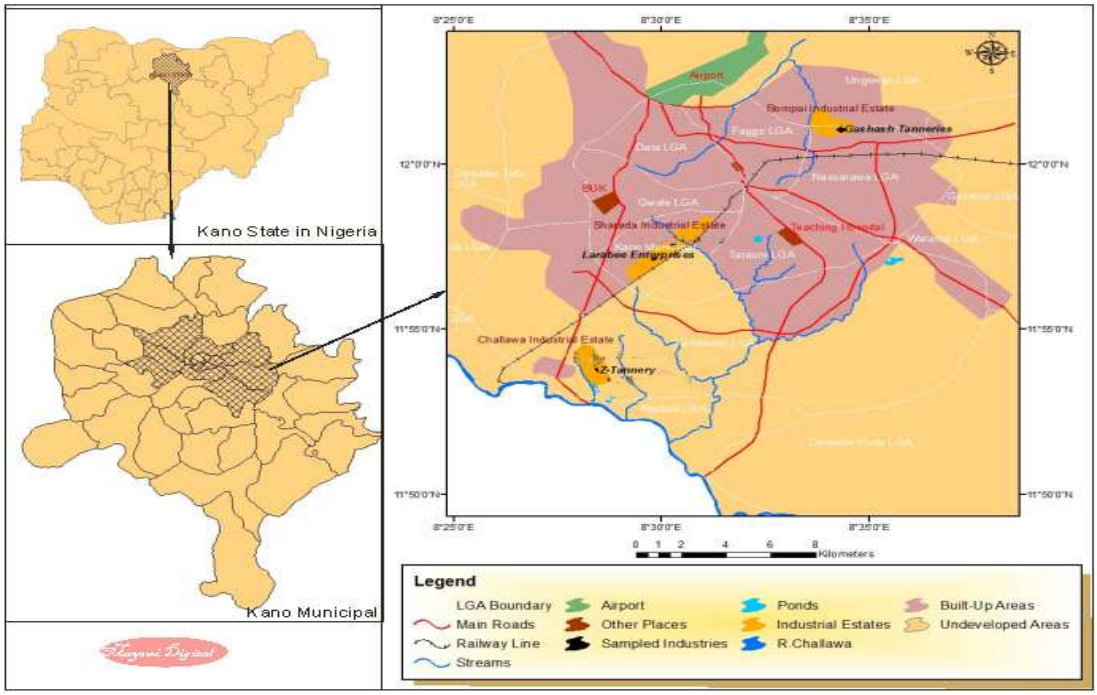

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

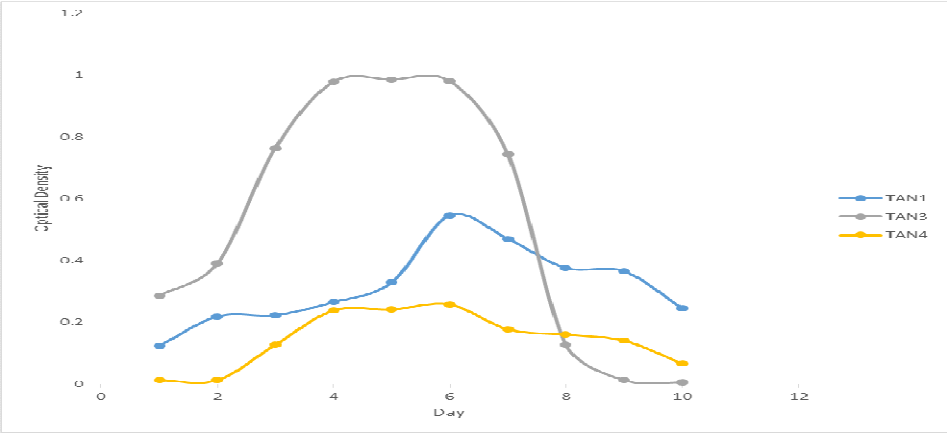

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

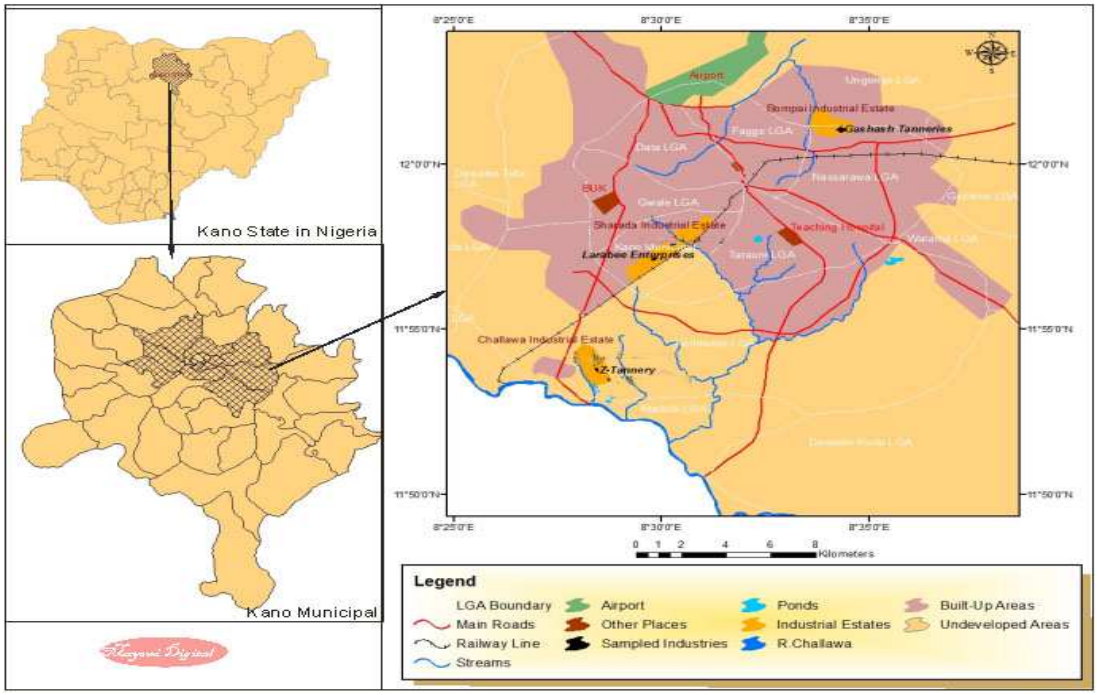

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

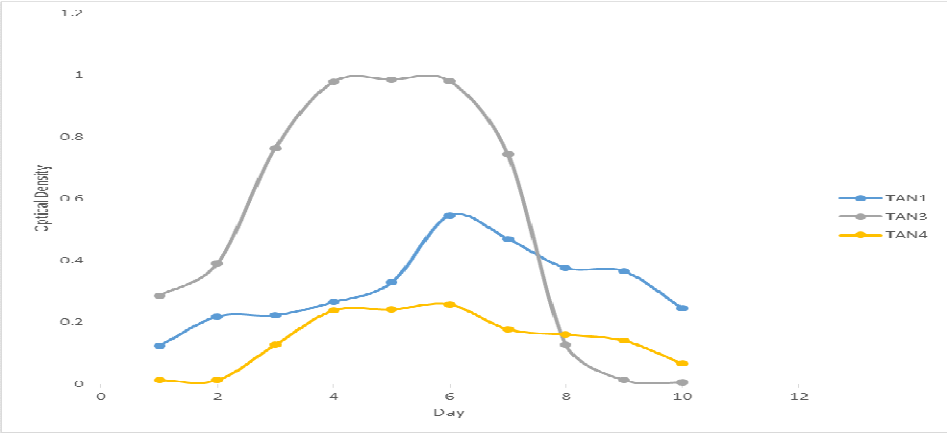

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

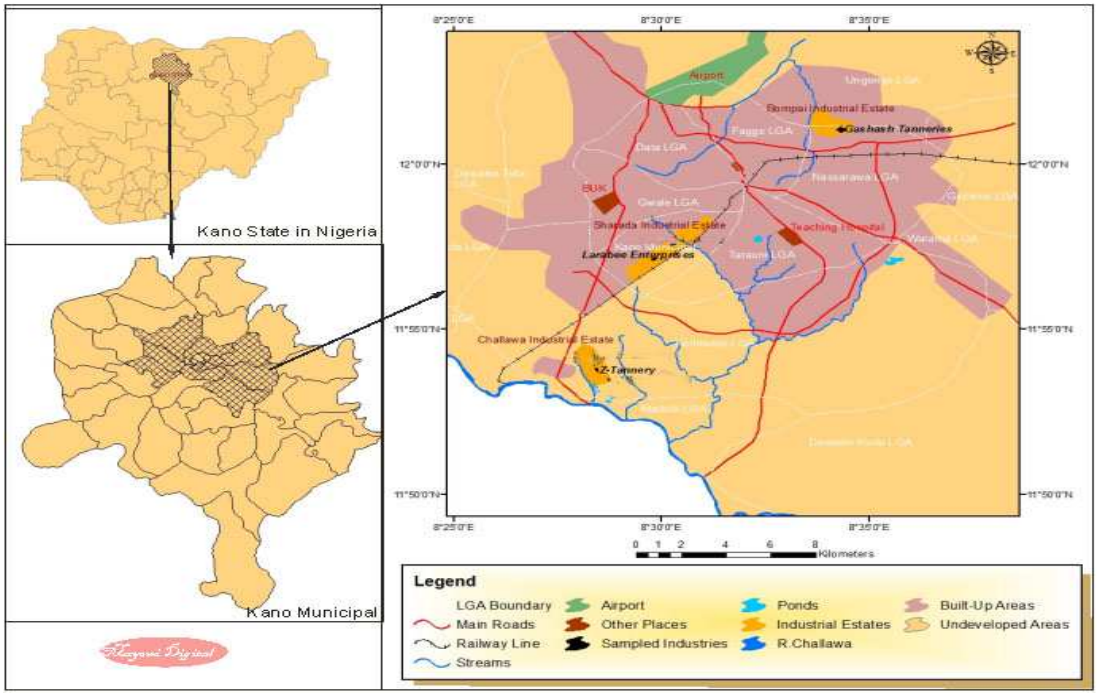

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

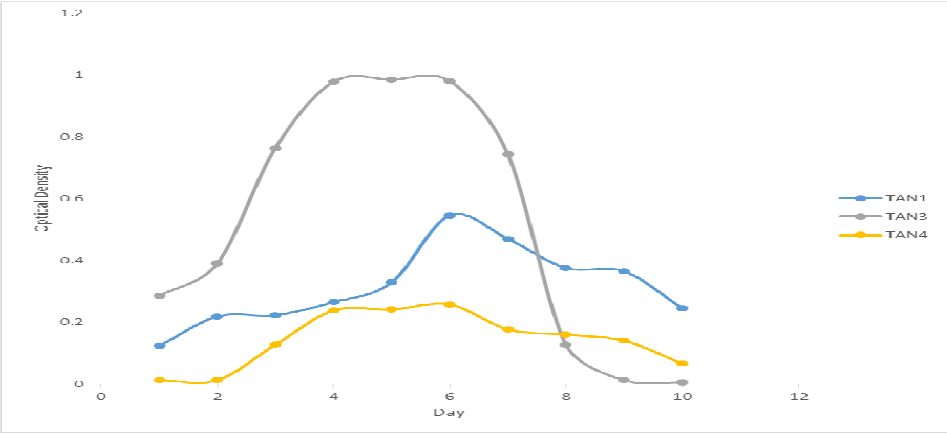

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

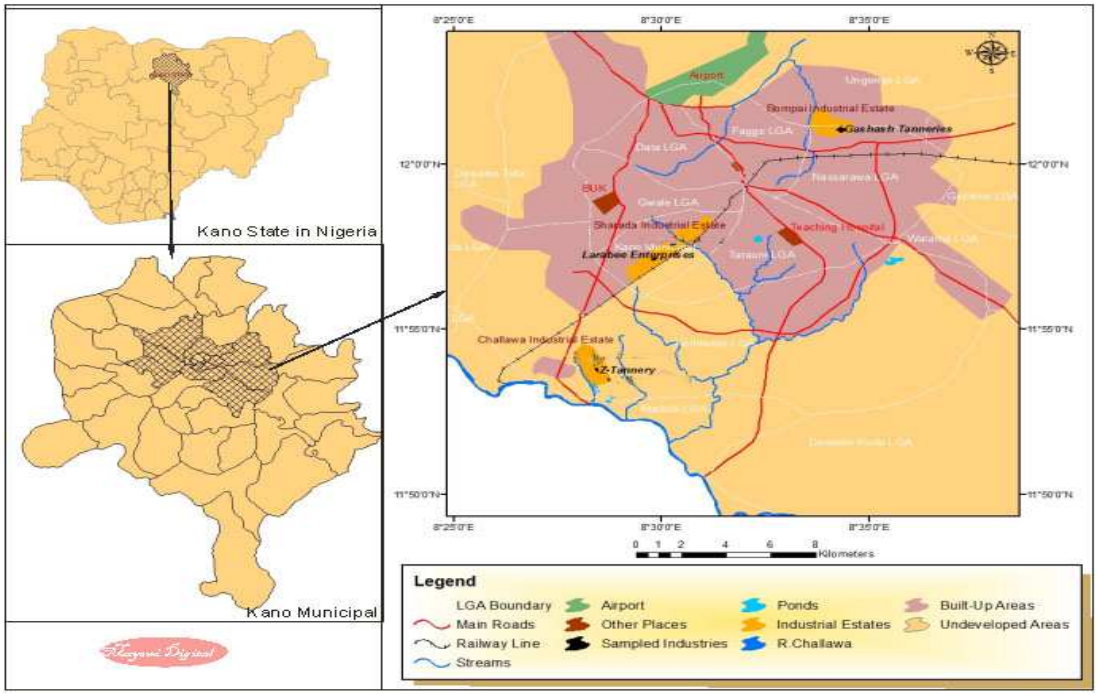

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

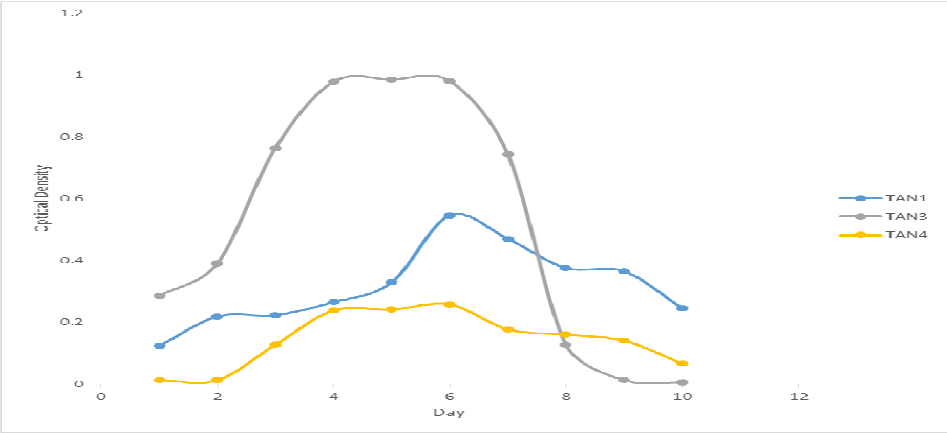

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

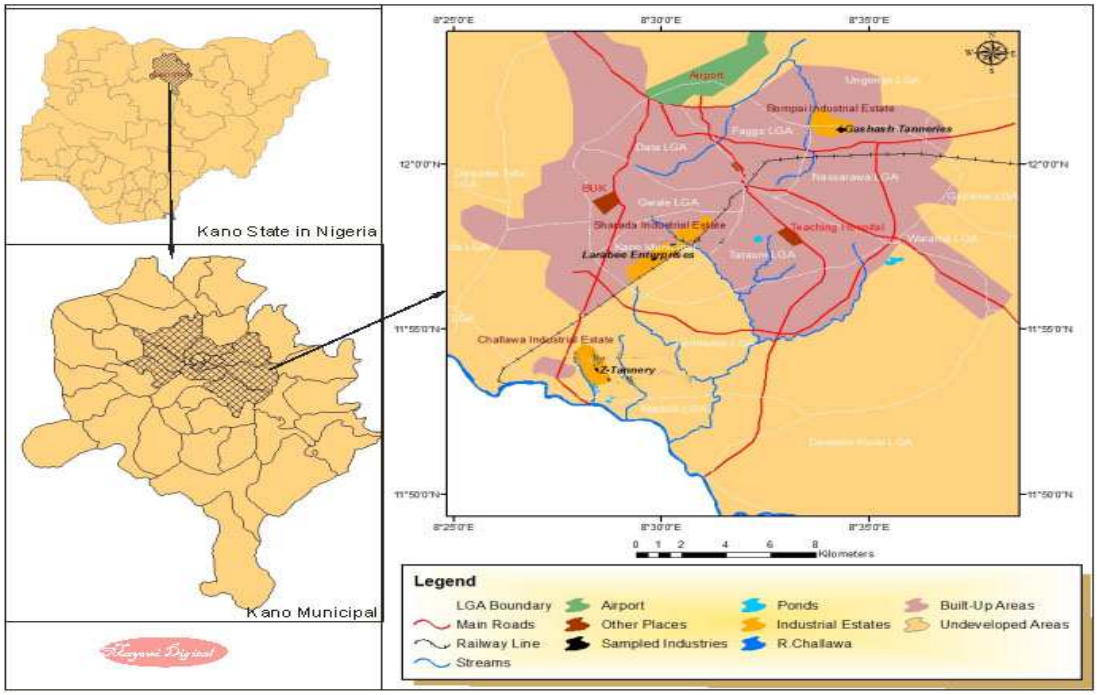

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

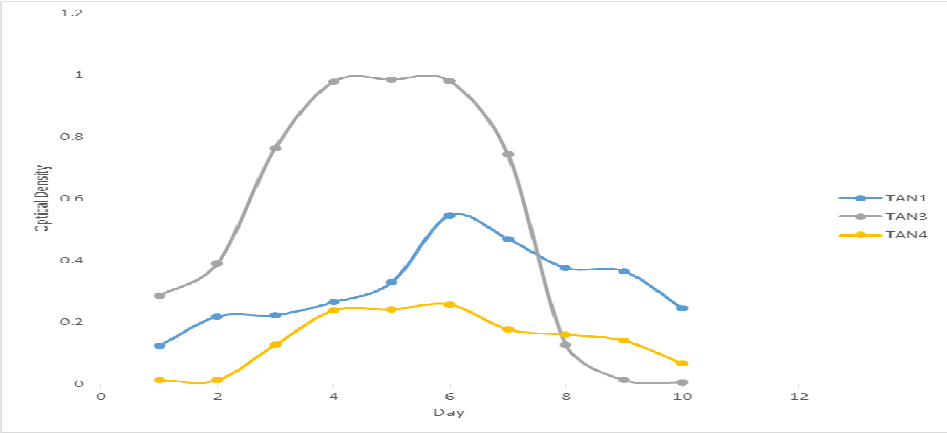

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

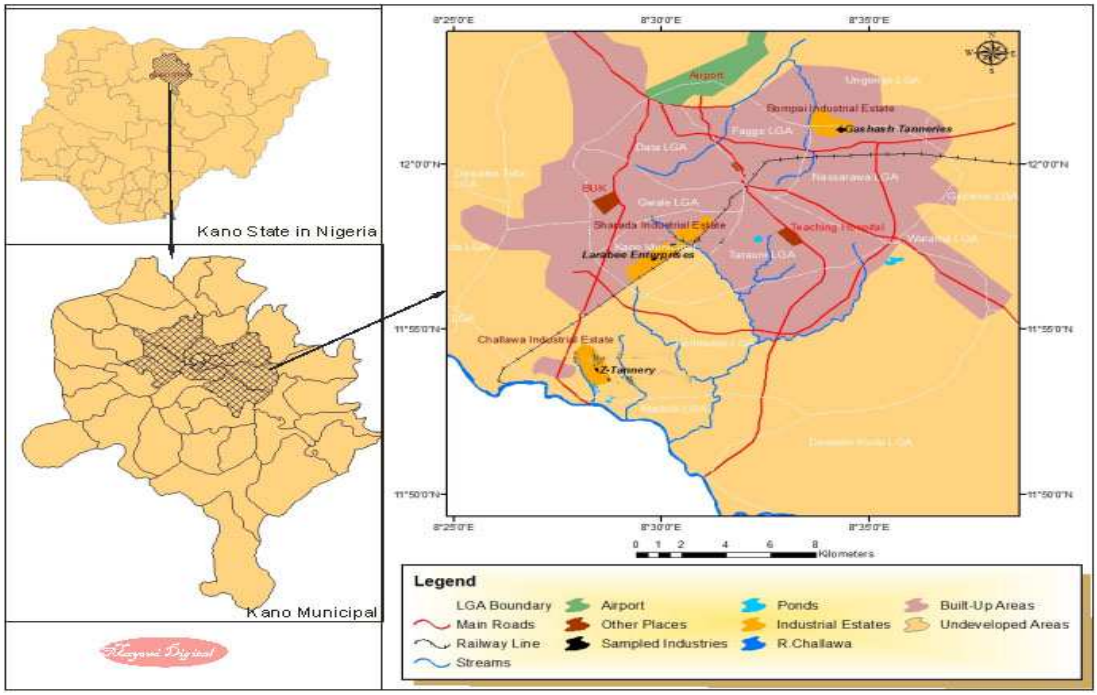

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

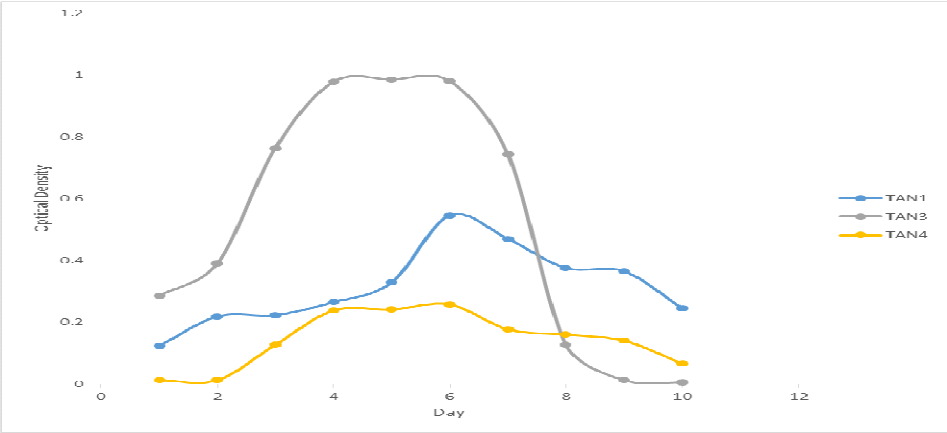

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

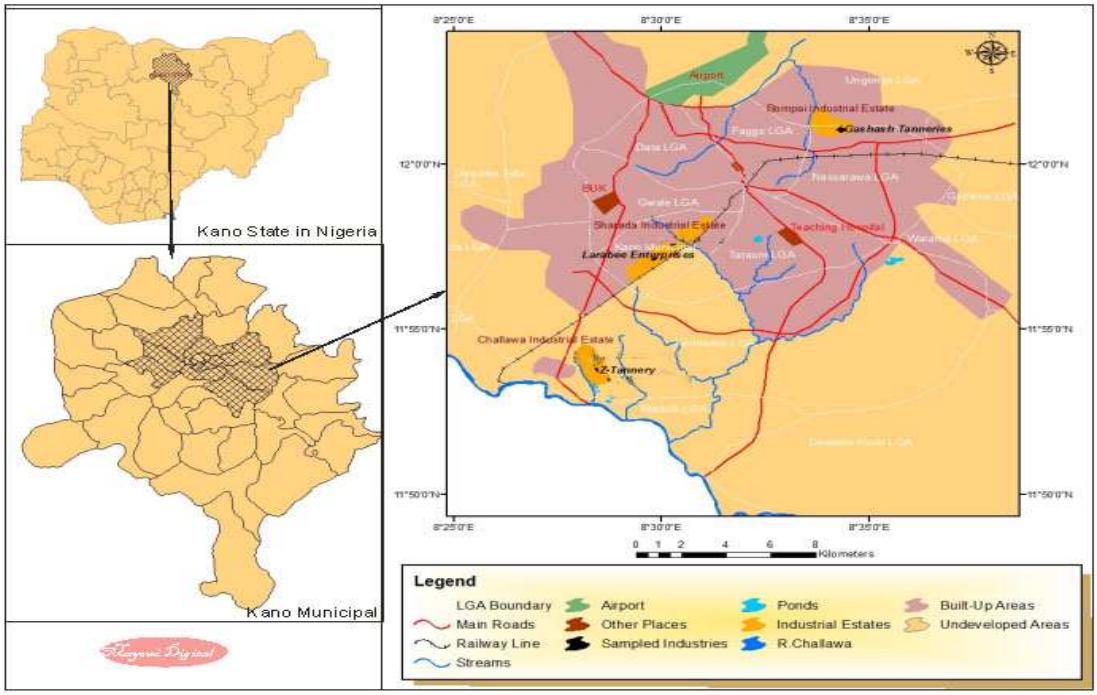

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

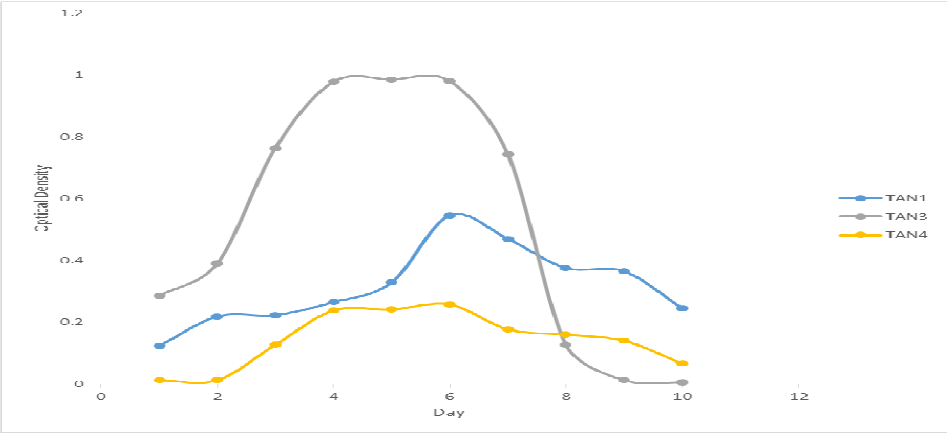

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

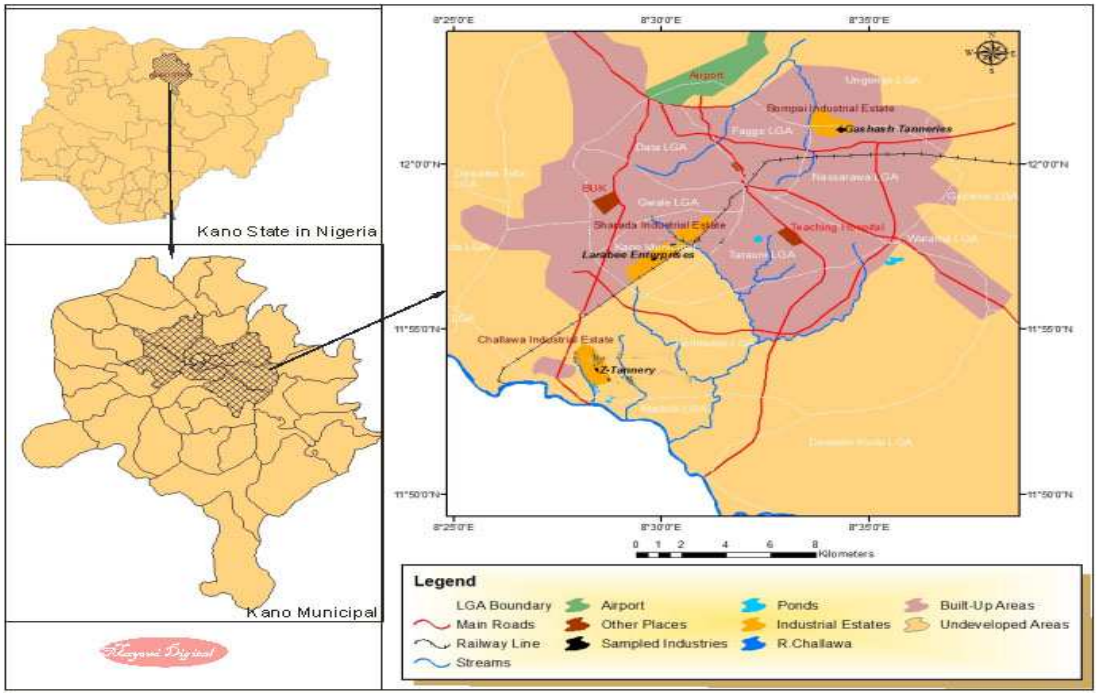

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

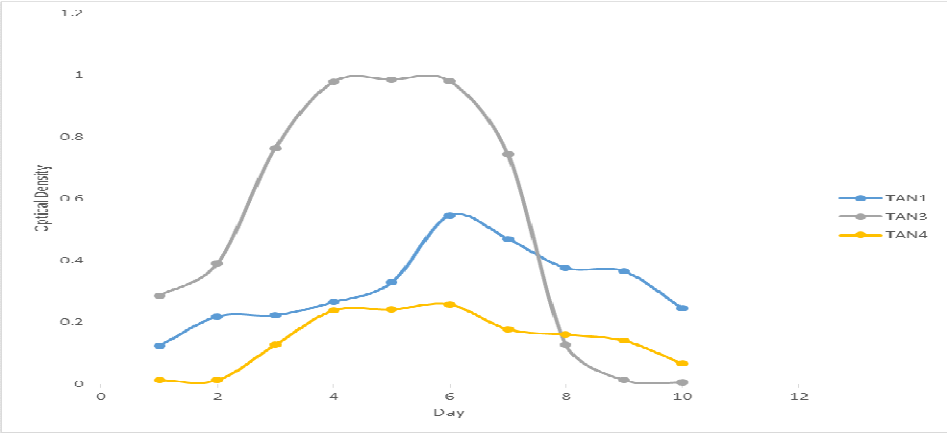

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

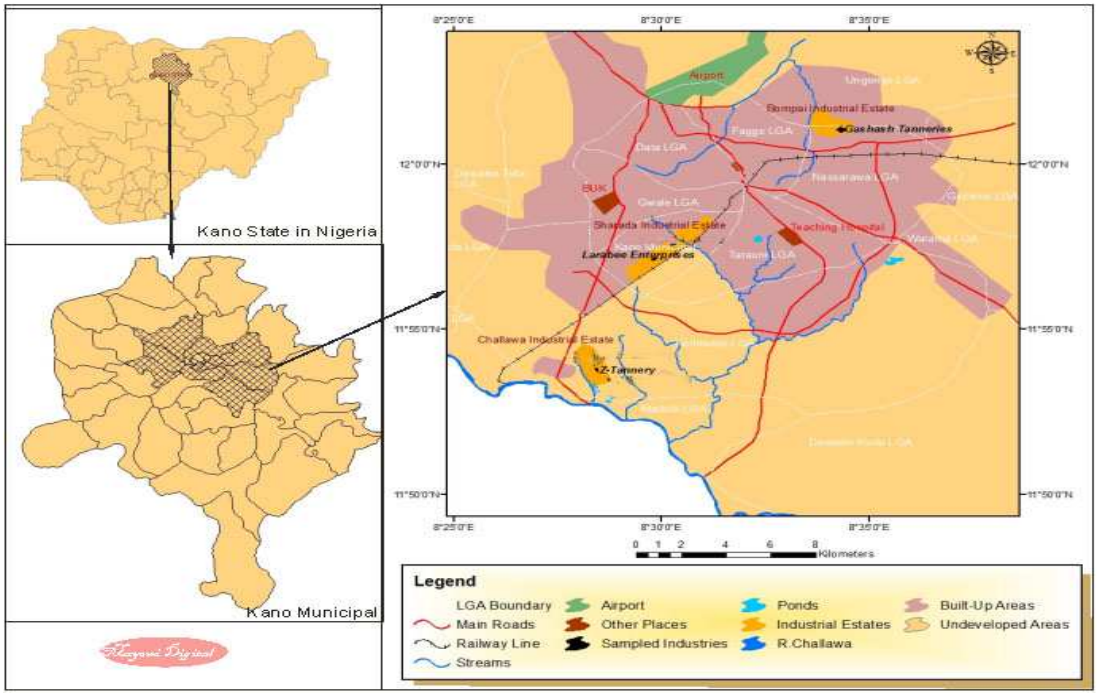

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

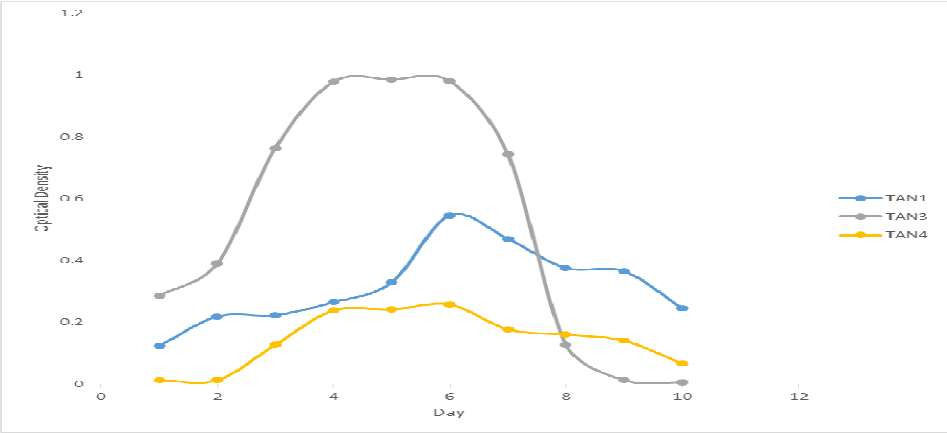

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

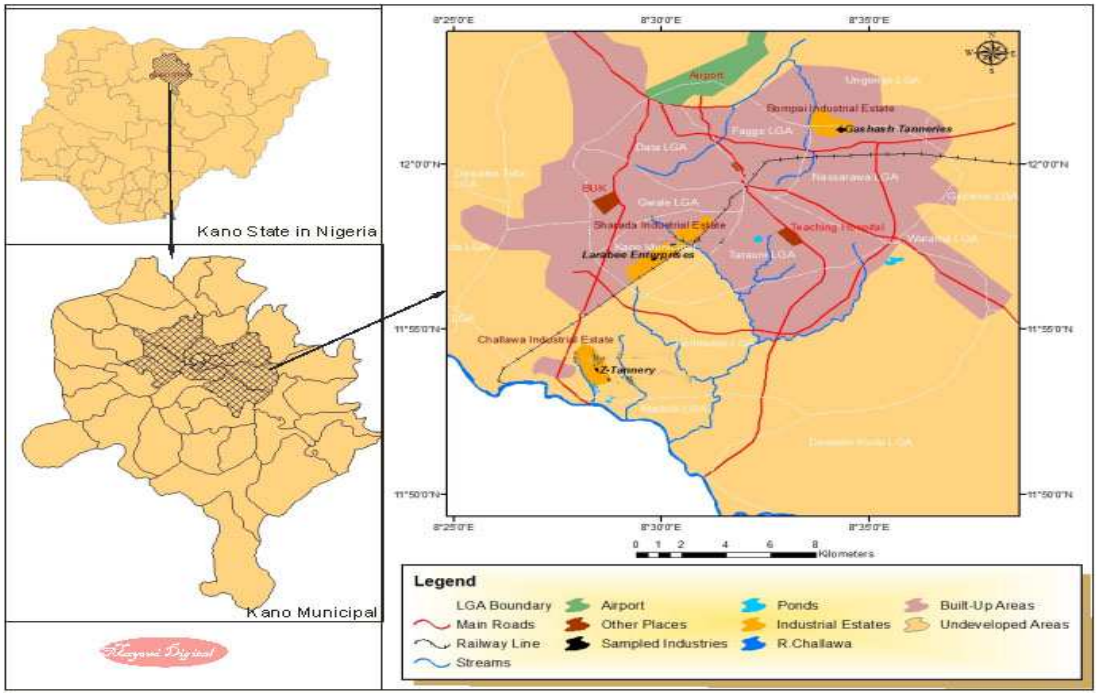

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

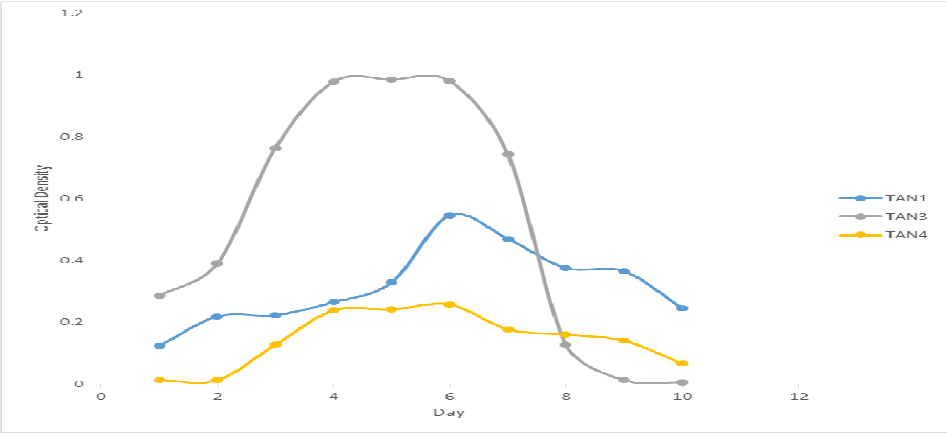

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

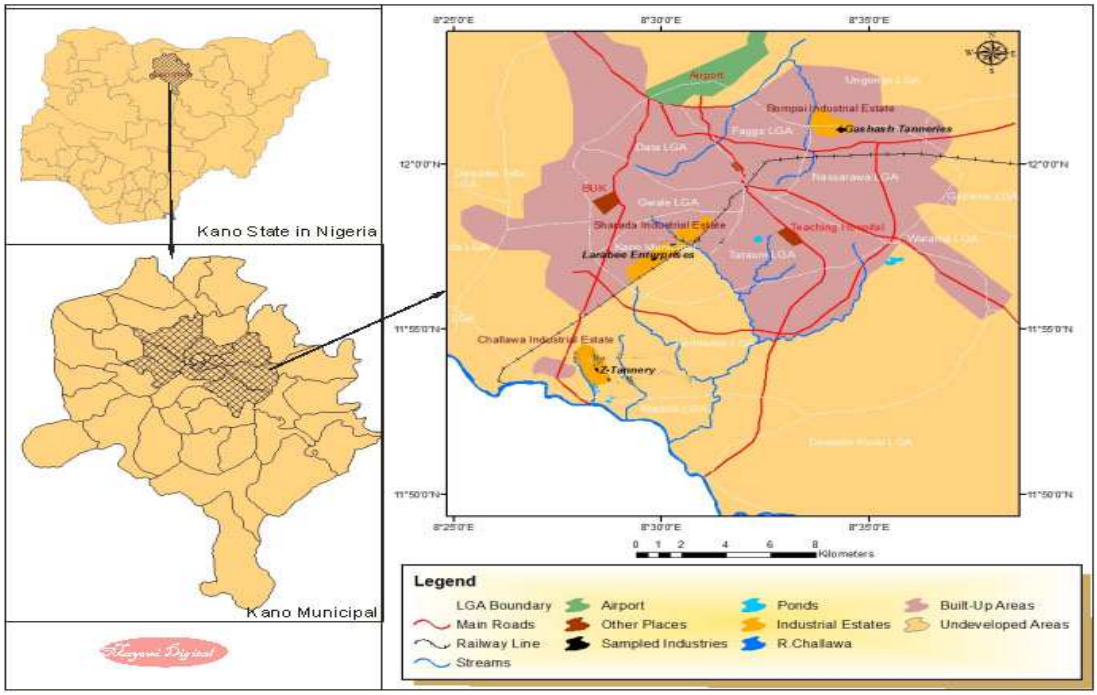

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

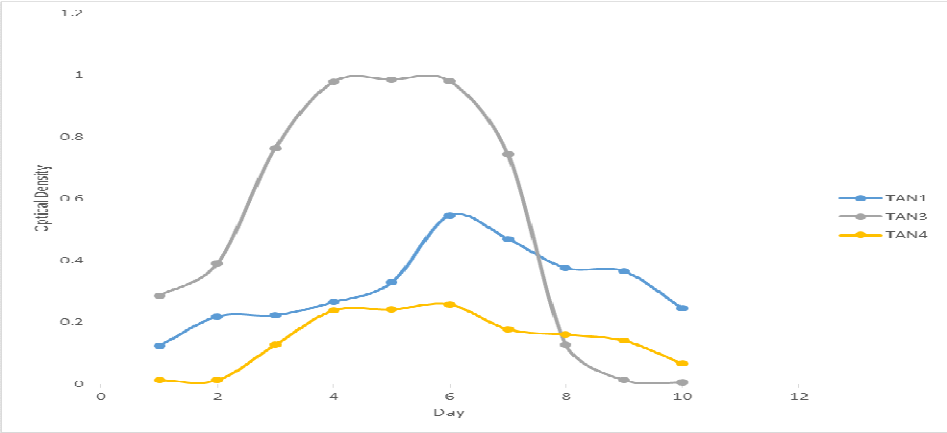

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

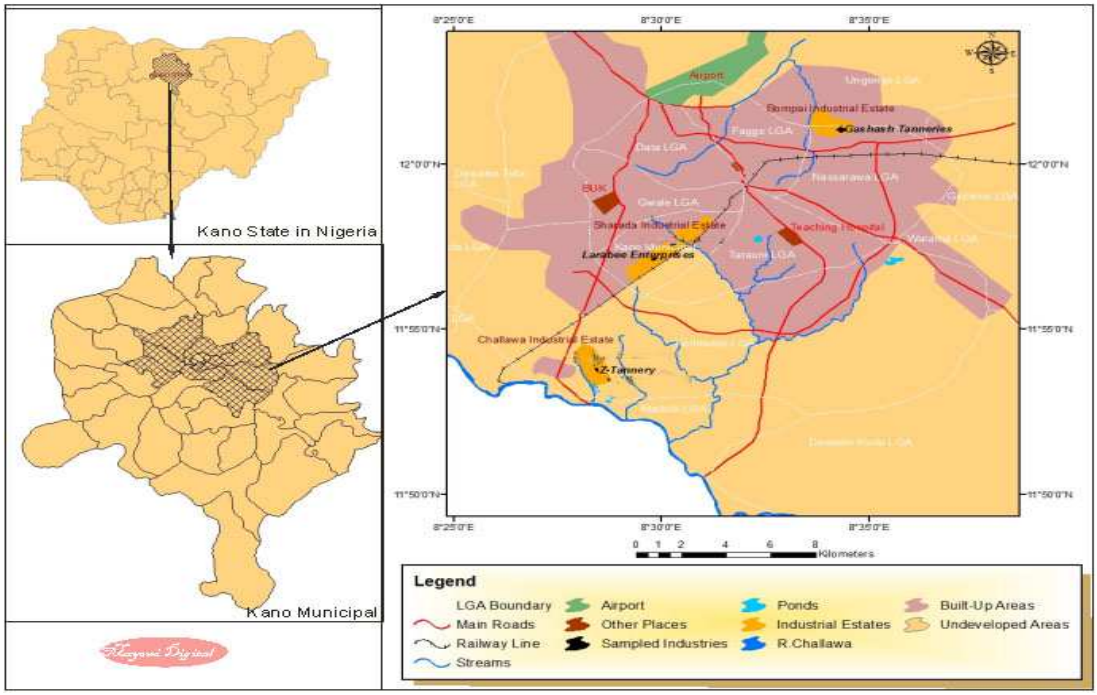

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

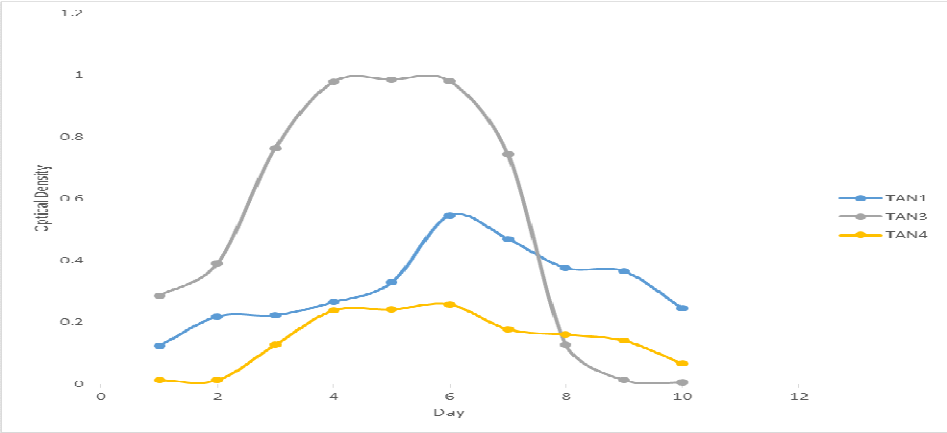

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

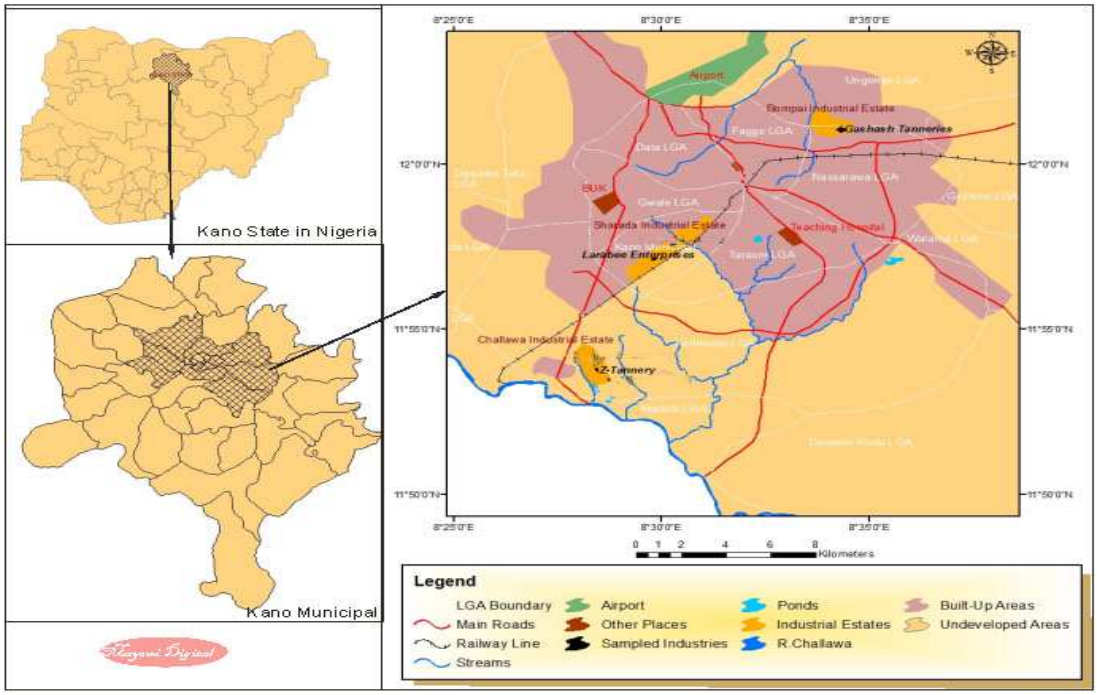

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

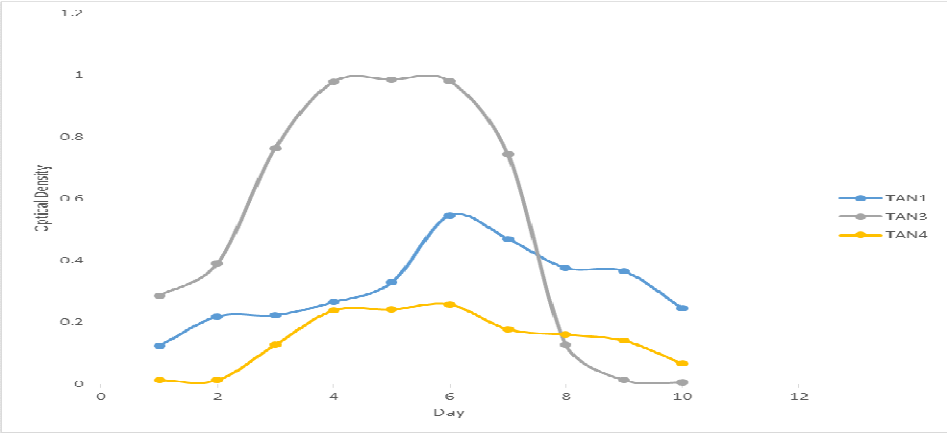

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

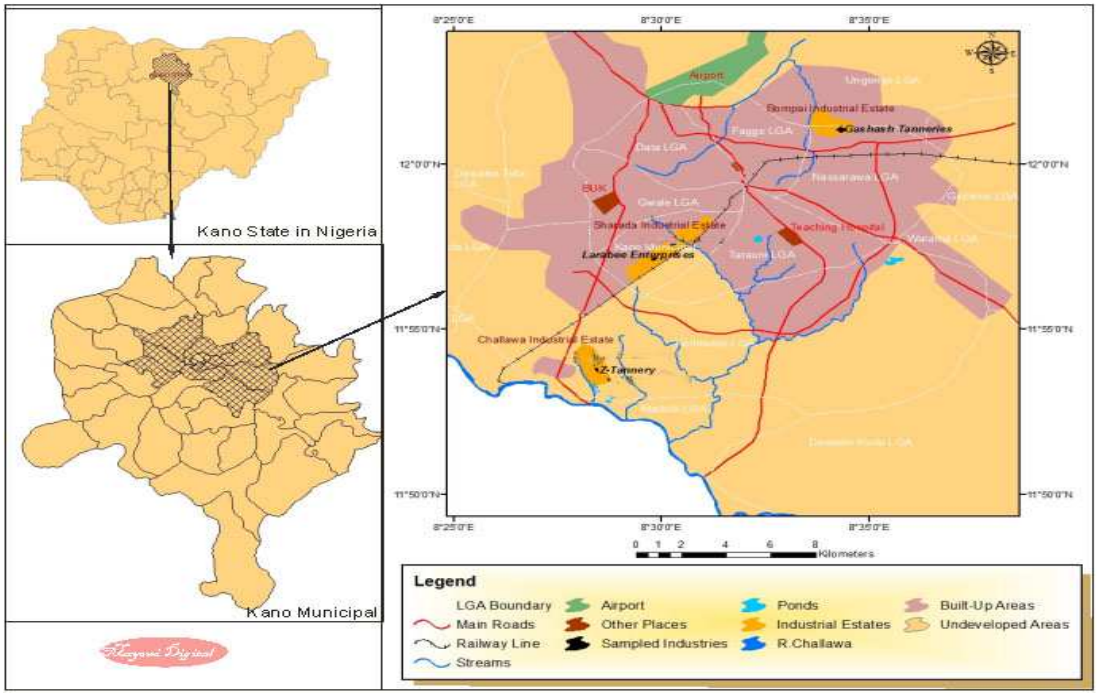

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

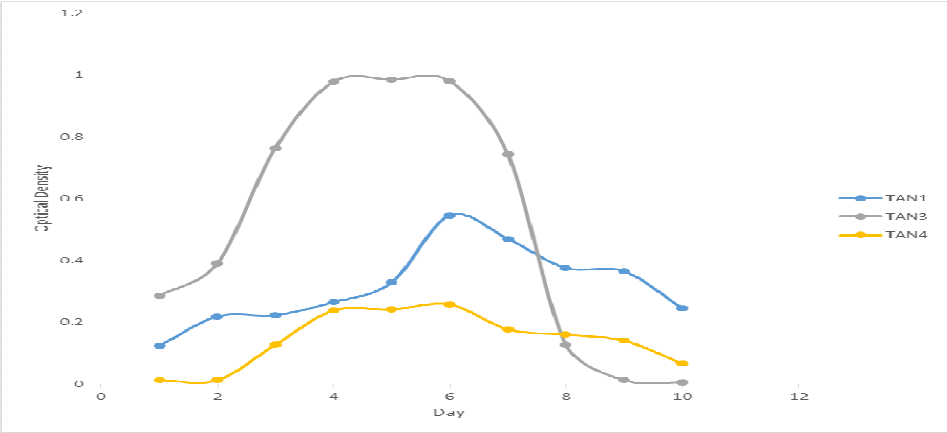

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

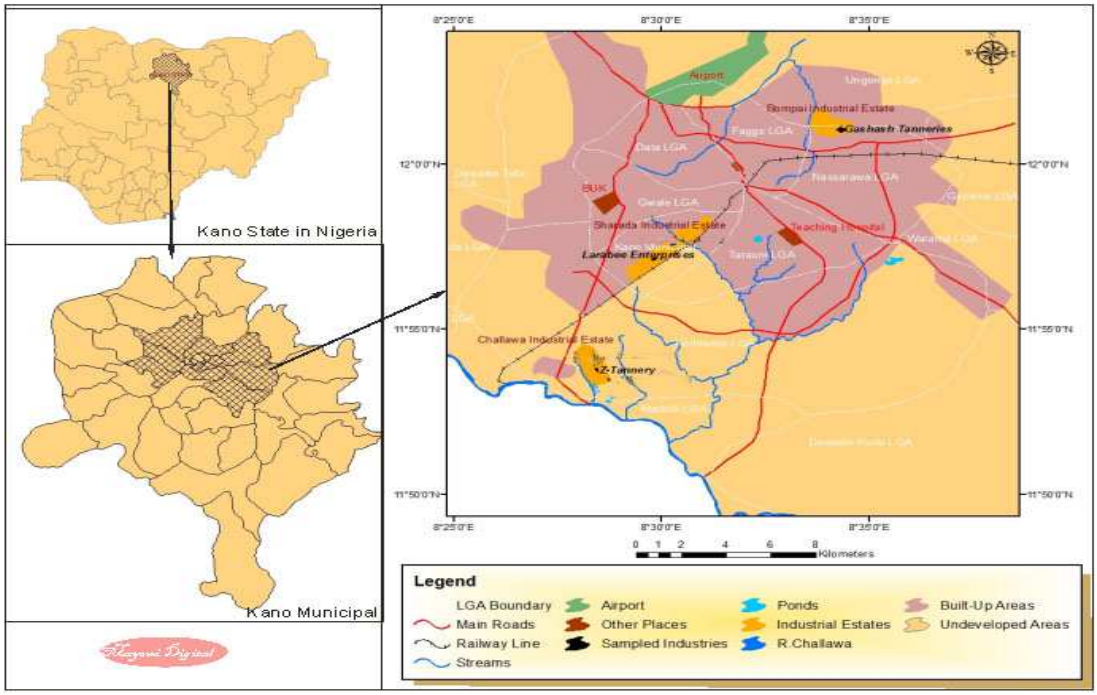

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

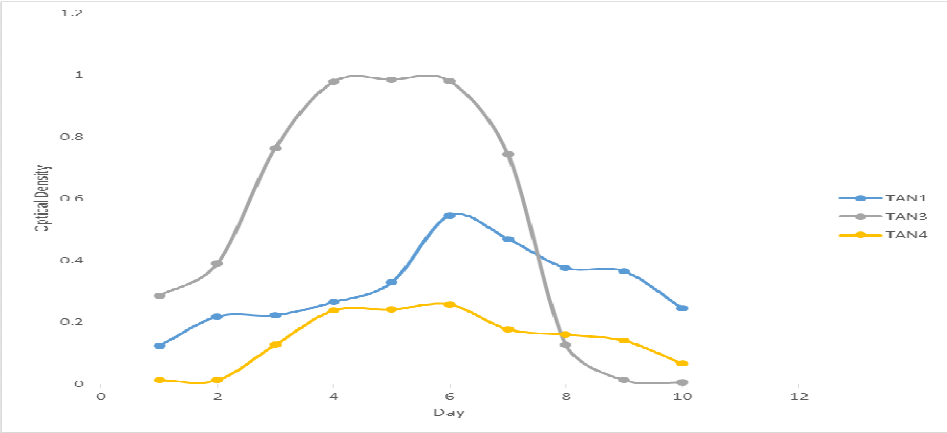

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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BIODEGRADATION POTENTIAL OF IMMOBILIZED BACTERIA IN THE TREATMENT OF TANNERY INDUSTRIAL EFFLUENTS FROM INDUSTRIAL ESTATES IN KANO STATE, NIGERIA

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ABSTRACT
Industrial Effluents Samples from Gashash Tanneries (TAN1) in Bompai Industrial estate, Larabee Tannery Industry (TAN2) in Sharada Industrial estate and Z Tannery Industries (TAN3) in Challawa Industrial estate, Kano State, Nigeria were collected over a period of six months (August 2017 to January 2018) for assessing the biodegradation potentials of bacteria in the treatment of organic pollutants within the effluents. Bacteria were isolated from the effluents and immobilized on agar-agar. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS) and Total Dissolved Solids (TDS) gives the following results; temperature (°C) ranged (26.38±3.81-30.33±3.79); pH (5.35±1.57-9.00±0.78); BOD (13.85±6.42-38.75±16.20); COD (1406±208-3532±1373); SS (208±235-780±739) and TDS (266±253-5276±2971). No statistical differences ($p \leq 0.05$) was observed for all the results among the different industries. The bacterial isolates were identified as Neisseria spp, Bacillus cereus, and Staphylococcus aureus, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluent with the different masses of the isolated bacteria, the mean level of BOD was found to range as (0.55±0.36-6.92±5.49); COD (ND-3134±1595); SS (18±022-898±672) and TDS (4±002-83±078). The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.
Keywords: Biodegradation, bacteria, effluent, immobilization, tannery.

INTRODUCTION

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit very high value of chromium (Cr), Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (Mohammed *et al.*, 2001). Tanning is the process, which One ton of skin generally leads to the production of 20 to 80 m^3 of turbid and foul-smelling converts the protein of the rawhide or skin into a stable material, which will not putrefy and is suitable for a wide variety of end applications (Elsheikh, 2009). The highly polluting chromium is the most commonly used tanning material producing leather that is more flexible and pliable than vegetable-tanned leather and does not discolor or lose shape in water as drastically as vegetable-tan (Elsheikh, 2009). Tannery effluent is among the most hazardous industrial pollutants due to its huge organic and inorganic load, which is highly toxic to human life and the environment (Kongjao *et al.*, 2008). wastewater including chromium (100–400 mg/l), sulfide (200–800 mg/l), high levels of fat and

other solid wastes, and notable pathogen contamination as well as pesticides added for skin conservation during transport (Elsheikh, 2009). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (Omoleke, 2004; Singh *et al.*, 2008). It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1995). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food) (Verheijen *et al.*, 1996). Tanneries generate wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), high concentrations of suspended solids (SS), and tannins as well as chromium (Zahoor and Abdul, 2009).

Being heterogeneous and composed of a wide variety of compounds, it is very difficult to select a unique direct method for estimating the biodegradability of organic contents and bio-kinetic parameters for a wastewater sample (Rajor, 2004). For this purpose, some indirect estimation such as determination of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are applied as common laboratory investigations [9]. During retanning procedures, synthetic tannins (Syntan), oils and resins are added to form softer leather at varying doses (Munz *et al.*, 2009). One of the refractory groups of chemicals in tannery effluents derives mainly from tannins (Ramasami *et al.*, 2004). Syntans are characterized by complex chemical structures, because they are composed of an extended set of chemicals such as phenol-, naphthalene-, formaldehyde- and melamine-based syntans, and acrylic resins (Beem, 1994). Organic pollutants (proteic and lipidic components) are originated from skins (it is calculated that the raw skin has 30% loss of organic material during the working cycle) or they are introduced during processes (Hugo Springer, 1994).

Many conventional processes such as oxidation, chemical and biological processes were carried out to treat tanneries wastewater (Ebtesam *et al,* 2013). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness (Noorjahan, 2014). Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time (Ranen and Sharadinadra, 2009). Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism (Bouwer and Zehnder, 1993). Bacteria, protista and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism (Bouwer and Zehnder, 1993). The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Ranen and Sharadinadra, 2009). An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode (Ranen and Sharadinadra, 2009). It has been observed that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation (Ranen and Sharadinadra, 2009). Even though there are numerous physical and chemical methods employed in the disposal of wastes the advantage in using bacterium is that they play a key role in the reduction of COD, BOD, total protein, total tannin and total phenol (Saravanan and Saravanan, 1998)

Baba *et al.* (2020) studied the bioremediation potential of immobilized *corynebacterium kutsceri* in the Treatment of tannery industrial effluent from Challawa Industrial Estate, Kano State, Nigeria. The aim of the work is to study the reduction in the level of the contaminants through the process of bioremediation using the isolated bacteria. Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency (Baba *et al.*, 2020).

MATERIALS AND METHODS

Study Area

This study was carried out in Bompai, Sharada and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30′N and 8°30′E and Longitude 11°5′N and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density (Dan'Azumi and Bichi, 2010). Many researchers have studied biodegradation of tannery effluent using microorganisms. However, limited literature is available on the biodegradation of tannery effluent in Kano industrial estates using

immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of tannery effluents in the industrial estates.

Sample Collection

Effluents were collected from the Tannery Industries from Bompai, Challawa and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected over a period of six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, TSS, TDS, COD, BOD levels were determined before treatment (Pre-treatment determination) and ten days after treatment (Post-treatment determination) as described in APHA (1989) standard methods. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS Salinometer. BOD was determined as described by the standard method (APHA, 1992). COD and SS were determined using DR/2010 HACH portable data logging spectrophotometer (DWAF, 1992)

Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (1989). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et al.* (2011).

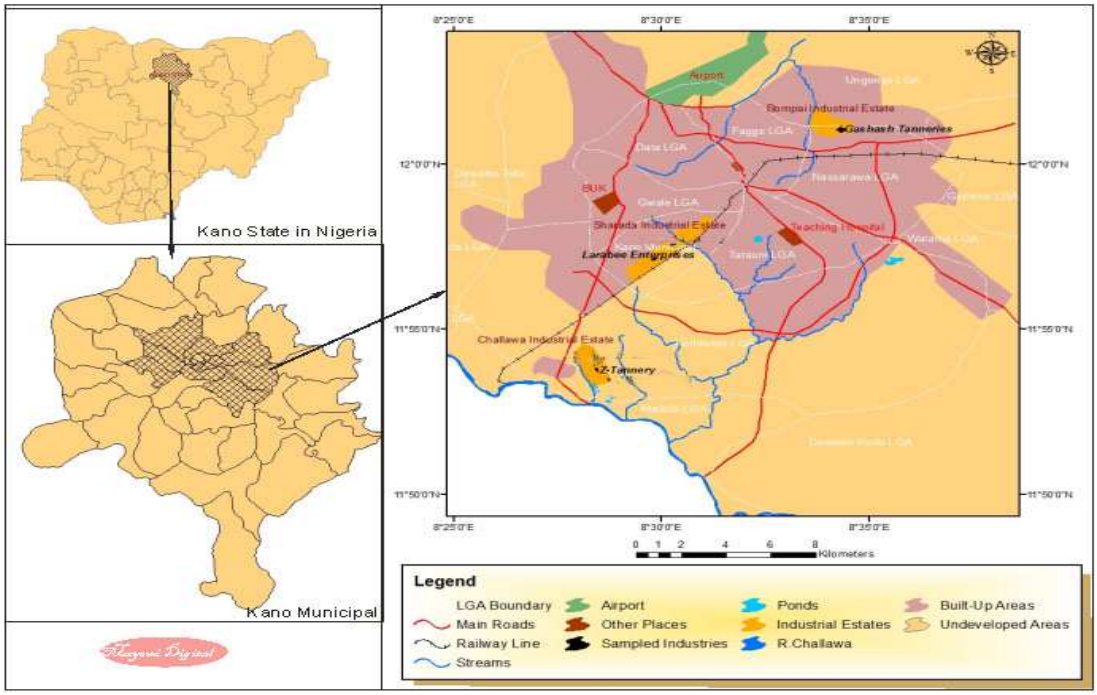

Fig. 1 Map showing the study areas

Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45°C (Ajao *et al*., 2011). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used (Ajao *et al*., 2011). Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes (Margesin and Schinner, 2001). Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber. The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at a temperature 30 °C and speed of 60 rpm. The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, and BOD, (Post-treatment determination) for the different grams of bacteria to evaluate and compare the biodegradation potential. (Baba *et al*., 2020).

Statistical Analysis

The data were represented as Mean± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Physico-chemical parameters in the Industrial Effluents before the Biodegradation.

Results of the Physico-chemical parameters in the industrial effluents before the Biodegradation is shown in table 1. The mean temperatures (ºC) observed in TAN1, TAN2, and TAN3 samples were 28.07±0.65; 27.77±0.64 and 26.38±3.81 respectively. The order of the mean temperature of the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The temperature observed at TAN1, TAN2, and TAN3 samples were found below the WHO (35ºC) and NESREA (40ºC) limits. The low values of temperature might be due to the processes used at the time of sampling. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of temperature among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of temperature observed in this present study are less than those observed by Akan *et al*. (2007), Akan *et al.* (2009) and Baba *et al*. (2020).

The mean level of pH observed in TAN1, TAN2 and TAN3, samples were 7.77 ± 2.93; 8.35±0.28 and 7.52±0.76 respectively. The order of the mean pH of the samples from the three industries can be arranged as TAN2> TAN1>TAN3. The pH of the samples falls within the WHO (7.0-8.5) and NESREA (6-9) standard limits. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of pH among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. Maheshwari *et al*. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was lower than that observed in the present study. The pH in the effluents from the tannery industries in Kano and Kaduna were reported to be 7.64 and 6.89, respectively (Akan *et al*., 2007; Mohammed *et al*., 2017). The average values of pH observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

The mean level of SS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 374±124; 358±335 and 780±739 respectively. The order of the mean SS in the samples from the three industries can be arranged as TAN3>TAN1>TAN2.

The SS observed in the samples were far above the recommended standard limits of regulating bodies WHO (30 mg/l) and NESREA (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The average values of SS observed in this present study are less than that observed (3700±122 mg/l) by Akan *et al*. (2009) for tanneries in Kano. Also, the average values of SS observed in this present study are less than that observed by Mohammed *et al.* (2017) and Baba *et al*. (2020) with the exception in TAN3.

The mean level of TDS (mg/l) observed in TAN1, TAN2, and TAN3 samples were 3941±3703; 3300±1714 and 2653±1240 respectively. The order of the mean TDS in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The TDS observed in the samples were far above the recommended standard limits of WHO (250 mg/l) and NESREA (500 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. TDS in the effluents from the tannery industries in Kano, Nigeria was reported to be 1281 mg/l (Akan *et al*., 2007). The average values of SS observed in this present study are less than those observed by Mohammed *et al*. (2017) and Baba *et al.* 2020)

The mean level of COD (mg/l) observed in TAN1, TAN2 and TAN3 samples seasons were 2372±938; 1406±208 and 3532±1373 respectively. The order of the mean COD of the samples from the three industries can be arranged as TAN3>TAN1> TAN2. The COD observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (40 mg/l) and NESREA (40 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) in COD among the industries. This might be due to similar tannery activities involved in the tannery industries as at the time of sampling. The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. In comparison, the average values of COD observed in this present study were higher than that observed by Mohammed *et al.* (2017) but lower than that observed by *Baba et al*. (2020).

The mean levels of BOD (mg/l) observed in TAN1, TAN2 and TAN3 samples were 13.85±6.42; 19.46±0.50 and 17.13±3.14 respectively. The order of the mean BOD in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The BOD observed in TAN1, TAN2 and TAN3 samples were found below the recommended limits of NESREA (200 mg/l) but above WHO (10 mg/l). Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of BOD among the industries. This might be due to similar tannery activities involved in the tannery industries at the time of sampling. The low level of BOD recorded in this study is an indication of the low level of biodegradable organic solids in the effluent. The average values of BOD observed in this present study were lower than those observed by Mohammed *et al*. (2017) and Baba *et al*. (2020).

Table 1: Mean Values ± S.D of Physico-chemical parameters of effluents from the Tannery Industries before Treatment.

Parameter	Tannery 1	Tannery 2	Tannery 3	a	b	c	d
Temperature(ºC)	28.07a±0.65	27.77a±0.64	26.38a±3.81		29.50±4.68	35	40
pH	7.77a ± 2.93	8.35a±0.28	7.52a±0.76	6.89	5.35±1.57	7.0-8.5	6.0-9.0
COD (mg/l)	2372a±938	1406a±208	3532a±1373	2.2	3106±2753	40	40
BOD (mg/l)	13.85a±6.42	19.46a±0.50	17.13a±3.14	1032	26.17±9.49	10	200
SS (mg/l)	374a±124	358a±335	780a±739	501	562±482	30	10
TDS (mg/l)	3941a±3703	3300a±1714	2653a±1240	532.7	444±507	250	500

The values given in the table above are means of 6 replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organisation. NESREA: National Environmental Standard and Regulatory Enforcement Agency. a = Mohammed *et al.(*2017), b = Baba *et al*. (2020), c = WHO (2006), d = NESSRA (2009)

Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth. The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former.

The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2 and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decrease as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

Table 2: Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		**TAN1**	**TAN2**	**TAN3**
Morphological characteristics	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
Biochemical characteristics	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Manitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	*Neisseria* spp	*Bacillus cereus*	*Staphylococcus aureus*

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

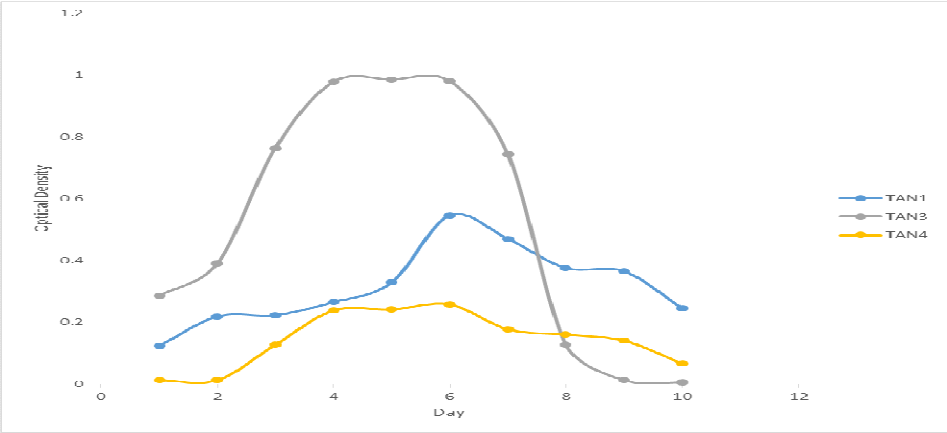

Fig. 2 Growth rates of the isolates in the effluents from the Tannery Industries

Physico-chemical Parameters in the Industrial Effluents after the biodegradation.

Table 3 shows the mean results of the physicochemical parameter before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table 4 shows the mean results of correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

The mean values (mg/l) of the SS after the bioremediation varies between 243±45 and 898±672. The mean concentration (mg/l) of SS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. The SS in the samples fluctuates up and down after the bioremediation process when compared with the SS of the raw samples before the bioremediation. The increase in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The increase in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. The relative potential or efficiency of the different masses of the bacteria in remediating SS in TAN1 samples was in the order 25 g>20 g>15 g>10 g>5 g. For TAN2 and TAN3 samples, the order was 25 g>20 g>15 g>10 g>5 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of SS among the masses in the respective industries. Positive and significant correlations exist between the masses of bacteria and Suspended Solid (SS). This showed that there is general increase in the levels of the SS as the masses of the immobilized bacteria increases. TAN3 (90%) and TAN1 (80%) showed a very high correlation with the masses of the bacteria while TAN2 (61%) showed a very low correlation.

The mean values (mg/l) of the TDS after the bioremediation varies between 46±11 and 83±78. The mean concentration (mg/l) of TDS remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the TDS of all the samples after the bioremediation process compared with the TDS of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating TDS in TAN1 and TAN3 samples was in the order 5 g>10 g>15 g>20 g>25 g. For TAN2 samples, the order was 20 g>10 g>25 g>15 g>5 g. Statistical analysis shows that there is no significant difference ($p<0.05$) between the mean values of TDS among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Positive and significant correlations exist between the masses of bacteria and TDS with the exception in TAN2 (negative and insignificant correlation). The positive correlations showed that there is general increase in the levels of the TDS as the masses of the immobilized bacteria increases. TAN1 (96%) showed a very high correlation with the masses of the bacteria while TAN2 (47%) showed a very low correlation.

The mean values (mg/l) of the BOD after the bioremediation varies between 1.56±0.20 mg/l and 6.92±5.49 mg/l. The mean concentration (mg/l) of BOD remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the BOD of all the samples after the bioremediation process compared with the BOD of the raw

samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating BOD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>10 g>5 g, 25 g>15 g>5 g>10 g>20 g and 20 g>10 g>25 g>15 g>5 g respectively. Statistical analysis shows that there is significant difference ($p<0.05$) between the mean values of BOD among the masses in the respective industries. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and significant correlations exist between the masses of bacteria and BOD. This showed that there is general decrease in the levels of the BOD as the masses of the immobilized bacteria increases. TAN1 (94%) showed a very high correlation with the masses of the bacteria while TAN2 (4%) showed a very low correlation.

The mean values (mg/l) of the COD after the bioremediation varies between 250±154 and 3134±1595. The mean concentration (mg/l) of COD remediated by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the bacteria varies. There is a reduction in all the COD of all the samples after the bioremediation process compared with the COD of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating COD in TAN1, TAN2 and TAN3 samples were in the order 25 g>20 g>15 g>5 g>10 g, 25 g>20 g>15 g>10 g>5 g and 10 g>5 g>25 g>15 g>20 g respectively. Statistical analysis shows that there were significant difference ($p<0.05$) between the mean values of COD among the masses in the respective industries except for effluents treated with 25 g. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. Negative and insignificant correlations exist between the masses of bacteria and COD with the exception in TAN3 (positive and significant correlation). The negative correlations showed that there is general decrease in the levels of the COD as the masses of the immobilized bacteria increases. TAN2 (100%) showed a very high correlation with the masses of the bacteria while TAN3 (36%) showed a very low correlation.

Generally, there was an overall decrease in the concentration of these physicochemical parameters after the bioremediation using the different masses of the bacterial isolates. These might be due to the variations in the surface areas of the different masses of the immobilized bacteria. This is in line with the work of Jimoh *et al*. (2018) and Baba *et al*. (2020).

Table 3: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

P	IND	Before	After				
			5 g	10 g	15 g	20 g	25 g
COD	TAN1	2372±938	1708a±861	2045a±1205	845a±369	300a±167	250a±154
	TAN2	1406±208	1195a±208	1125a±384	1055a±317	956a±310	870ab±240
	TAN3	3532±1373	2374a±1344	1976a±1405	2757a±1266	3134a±1595	2614b±1105
BOD	TAN1	13.85±6.42	6.92a±5.49	6.42a±5.07	5.72a±5.35	4.62a±4.37	2.82ab±1.26
	TAN2	19.46±0.50	1.75b±0.22	1.73b±0.18	1.58a±0.16	1.91a±0.22	1.56b±0.20
	TAN3	17.13±3.14	4.24ab±0.77	3.29ab±0.37	4.11a±0.07	3.23a±0.91	3.33a±1.28
SS	TAN1	374±124	243a±45	471a±226	475a±182	492a±128	611a±217
	TAN2	358±335	460a±400	543a±414	544a±402	551a±414	554a±405
	TAN3	780±739	586a±594	758a±656	787a±676	861a±635	898a±672
TDS	TAN1	3941±3703	51a ±10	53a ±10	55a ±15	61a±20	63a±26
	TAN2	3300±1714	83a±78	47a ±20	48a ±22	47a ±17	48a±17
	TAN3	2653±1240	46a±11	55a±24	55a±25	58a±23	61a±28

Replicate= 6 (months)

Within the rows, for the same parameter, means with different alphabets are statistically different ($p<0.05$).

P = parameter, IND = Industries

Table 4: Correlation coefficient (r) between different masses of the bacteria and the physicochemical parameters.

Industries	Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
TAN1	COD	-0.9	82
	BOD	-0.97	94
	SS	0.90*	80
	TDS	0.98*	96
TAN2	COD	-1	100
	BOD	-0.21	4
	SS	0.78*	61
	TDS	-0.69	47
TAN3	COD	0.60*	36
	BOD	-0.6	37
	SS	0.95*	90
	TDS	0.94*	89

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Percentage reduction of the Parameters

Table 5 shows the percentage reduction of Parameters in industrial samples before and after the treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of COD ranged (14-89); BOD (50-80); SS (-32-35) and TDS (98-99). In TAN2 samples, the percentage decrease (%) of COD ranged (15-38); BOD (90-92); SS [-28-(-55)] and TDS (97-98). In TAN3 samples, the percentage decrease (%) of COD ranged (11-44); BOD (76-81); SS (-15-25) and TDS (98). The percentage increase in the levels COD, BOD and TDS might be due to the increase in the surface area of the different masses of the immobilized bacteria. However, the percentage decrease in the levels of the SS might be due to the aggregation of the TDS which are large enough to result into SS. The percentage decrease in the levels of the SS might be also due to the influence of the nutrients which was added into the effluents in order to make the microorganisms more active and viable for fast degradation of organic contaminants in the effluent. This is in line with the work of Jimoh *et al*. (2018) in which the concentration of the SS increase after the bioremediation of effluents.

Sreemoyee and Priti (2013) assessed and reduced several Physico-chemical parameters of dairy wastewater using *Niesseria sp.* and concluded that the species are well known to degrade organic compounds. This is in agreement with the current study in which the immobilized *Niesseria sp* degrade the organic contaminants as indicated by the BOD, COD and TDS.

Jimoh *et al*. (2018) observed that TSS of the effluents was increased after treatment with immobilized bacteria and concluded that it might be due to the biostimulation method adopted for the research.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan (2011). The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilised *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the organic pollutants with high potential as indicated by the percentage reduction of BOD, COD and TDS.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed (Gianfreda and Rao 2004). Ramesh and Singh (1993) reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* (2007) showing the higher reduction with permeabilized cells of *Ochrobactrum intermedium* strain SDCr-5.

The results revealed the isolation and identification of isolates with the potential for the reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5: Percentage reduction of the tested Parameters from the tannery industrial samples of the Immobilized Bacteria.

Industries	Parameter (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	COD	28	14	64	87	89
	BOD	50	54	59	67	80
	SS	35	-26	-27	-32	-63
	TDS	99	99	99	98	98
TAN2	COD	15	20	25	32	38
	BOD	91	91	92	90	92
	SS	-28	-52	-52	-54	-55
	TDS	97	99	99	99	99
TAN3	COD	33	44	22	11	26
	BOD	75	81	76	81	81
	SS	25	3	-1	-10	-15
	TDS	98	98	98	98	98

Percentage Reduction= (B-A)/B ×100%
A= Concentration of the parameter after treatment
B= Concentration of the parameter before treatment
+ = percentage decrease
- = percentage increase

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (Gianfreda and Rao, 2004; FuIlbrook, 1996; Russell *et al*, 2003; Kandelbauer *et al*., 2004). Immobilized cells have been used and studied extensively for the production of useful chemicals (Ohtake and Silver, 1994), the treatment of wastewaters (Chen *et al*., 2003; Wang *et al*., 2010). Although many workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

CONCLUSION

The samples contained variable levels of the physicochemical parameters. The results of the Analysis of variance revealed that, no statistical difference ($p < 0.05$) was observed for the temperature, pH, SS, TDS, BOD and COD among the three tannery industries before the treatment. The levels of some of the parameters (SS, TDS and COD) observed in the samples were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus,* and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is overall decrease in the levels of the parameters determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilised bacteria in the treatment of the respective effluents was in the order TAN2 (72%)>TAN1 (70%)>TAN3 (62%). Hence, the immobilized bacteria are having higher biodegradation potential for the treatment of the tannery effluents.

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