

Full Length Research Paper

Biosorption of hexavalent chromium (chromium (VI) ion from industrial tannery effluent using filamentous fungi on modified rice husk medium/potato dextrose medium (MRHM/PDM)

S. D. Emmanuel^{1*}, I. K. Adamu¹, A. Ejila¹, M. I. Ja'afaru², Y. Amos³, M. Abubakar¹ and O. Agbor¹

¹Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Nigeria.

²Moddibo Adama University (MAUTECH), Yola, Nigeria.

³Department of Microbiology, Kaduna State University (KASU), Kaduna, Nigeria.

Received 11 December, 2013; Accepted 6 May, 2014

An experimental effluent analysis was conducted in conjunction with bioremediation process from Mario-jose tannery industry, stream and reference water samples from well (Yandoka), respectively, were collected from the industrial areas. Physicochemical characteristic of these samples were determined viz; chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, temperature etc. The effluent values were mostly observed higher than the reference samples. Spectrophotometric method using 1,5 dipheyl carbazide as a colour developing reagent has been used to analyzed the chromium (VI) content in tannery effluent. *Aspergillus niger*, *Rhizopus nigricans* and *Penicillium* sp singly and in combination were used for the biosorption studies. Chi- square test analysis for fitness shows that the concentration of the media were (Fcal 0.05) for PDM and (Fcal 0.102) for MRHM varies significantly ($\alpha=0.05$). Hence, the absorptions ability was highly effective on MRHM than the PDM. From the experimental observation, it has been found that the level of chromium (VI) can be quantitatively reduced at the equilibrium pH range from 5.2-8.0.

Key words: Biosorption, chromium (VI), *Aspergillus niger*, *Rhizopus nigricans*, *Penicillium* sp., agricultural waste, 1,5 dipheyl carbazide.

INTRODUCTION

Rapid industrialization and increase in population contribute greatly to heavy metal pollution in the environ-

ment. Such high concentration of metals above the tolerance limits are found in aquatic environment

*Corresponding author. E-mail: davidsilas550@gmail.com.

Abbreviations: COD, Chemical oxygen demand; BOD, biochemical oxygen demand; SmF, submerged fermentation; SSF, solid state fermentation.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

(Tewaria et al., 2005). Pollution by heavy metals is one of the serious environmental threats as a result of various industrial discharges (Wang and Cheng, 2009). Hexavalent Chromium (Cr (VI)) is a by-product released into the aquatic environment by many industrial activities such as leather tanning, chrome plating, stainless steel welding, pigment production and nuclear weapon production (Gonzalez et al., 2003). Chromium exist in two oxides states: Cr (III) and Cr (VI) and the most toxic form is Cr(VI), which has been implicated in causation of liver damage, pulmonary congestion and oedema (Babu and Gupta, 2008). The maximum permissible limit of Cr (VI) by environmental regulatory agencies of different countries for industrial waste water varies from 0.05 to 0.1 mg/l (Bansal et al., 2009). The cumulative toxicity and environmentally detrimental effects of chromium have led researchers to continue to find ways for its removal from industrial (especially tannery) waste. The removal of this metal from waste water by conventional physio-chemical methods such as chemical, precipitation, chemical oxidation and reduction, ion exchange, filtration reverse osmosis etc have not been found to be very effective and are highly expensive especially when the concentration is less than 100 ppm (Yan and Viraraghavan, 2003). In recent years, bio-sorption of heavy metals by microbial cells has been recognized as a potential alternative to the existing technologies for removing heavy metals from industrial effluent (El-Sherif et al., 2008); accumulation of metals by microorganisms or their products has received more attention as the metal ion concentration lower than 10 mg^{-1} can thus be removed (Kapoor et al., 1995). These bio remedial process use either viable or nonviable cells to removed heavy metals ions from aqueous solutions (Kapoor et al., 1995) that bacteria, algae, yeasts and other fungi have been used successfully as adsorbing agents for heavy metal (Kumar et al., 1998).

Fermentation processes may be divided into two systems: submerged fermentation (SmF), which is based on the microorganisms cultivation in a liquid medium containing nutrients, and solid state fermentation (SSF), which consists of the microbial growth and product formation on solid particles in the absence (or near absence) of water; however, substrate contains the sufficient moisture to allow the microorganism growth and metabolism (Pandey et al., 2008). In recent years, SSF has received more interest from researchers since several studies have demonstrated that this process may lead to higher yields and productivities or better product characteristics than SmF. In addition, due to the utilization of low cost agricultural and agro-industrial residues as substrates, capital and operating costs are lower compared to SmF. The low water volume in SSF has also a large impact on the economy of the process mainly due to smaller fermenter-size, reduced downstream processing, reduced stirring and lower sterilization costs (Pandey et al., 2008). The use of biosorption tech-

nology using granular and powered activated carbon has been carried. However, prohibitive costs limit their extensive utilization especially in developing countries because of growing interest, the low-cost easily available and environment friendly agricultural residues have been tested as metal biosorbents and a number of agro based plant waste materials such as papaya wood (Saeed et al., 2005), and rice husk (Tarley and Arruck, 2004) coconut fibres, (Espinola et al., 1999) and black gram husk (Saeed and Iqbal, 2003) have been reported as potential biosorbents.

Parkia biglobosa can be found in a belt stretching from Atlantic coast in Senegal to Southern Sudan and Northern Uganda (Thiombiano et al., 2012), the tree currently exist within a wide range of natural communities but is most abundant in anthropic community places where cultivation is semi-permanent (Janick, 2008). Annual production of seeds in Northern Nigeria is estimated at around 200,000t, while the products of the trees are not common in international trade (Ntui et al., 2012). Yellow purple or yellow pericarp which contains the seeds is naturally "sweet", and is processed into a valuable carbohydrate food known as "Sikomu and dodawa" among the Yoruba and Hausa people of Nigeria, respectively (Olaniji, 2013). The yellow pericarp which is known as waste from *P. biglobosa* is naturally and highly rich in glucose (69%), while the most valuable parts of the locust bean are the seeds themselves which are high in lipid (29%), protein (35%) and carbohydrate (16%) and is a good source of fat and calcium for rural dwellers (Gbolagunte et al., 2003; Ntui et al., 2012) respectively.

The organic material such as *Tamarindus Indica* (Tsamia)/yellow pulp (*P. biglobosa*) dorowa respectively served as an alternative for the use of dilute acid (H_2SO_4 , HNO_3 and HCL) as pH adjustment in solution while the yellow purple from locally available (*P. biglobosa*) dorowa contained natural glucose that further boost the natural carbon source present in the rice husk for effective utilization of the substrate by the microbe in medium. The abundance of rice husk in most developing countries as well as its composition and cost effectiveness etc. makes it strong candidate to be used as an adsorbent for removal of many pollutants from aqueous solution. The current economic values of rice husk are less than the apparent cost of transportation and processing beneficial used (Foo and Hameed, 2009). Thus, rice husk has been used as an absorbance to remove heavy metal and fiber roughage in animal feed in a very minute quantity, if highly processed or modified with adequate energy source by combustion (Lin et al., 1995). In this case direct utilization of rice husk as metal biosorbent would be economically and practically useful by solid state fermentation.

This study is aimed at exploring the feasibility of rice husk using filamentous fungi strain for uptake of chromium (VI) solution using 1,5-diphenyl carbazide.

The significance of the spectrophotometric analyzer in a particular adsorption is economically feasible alternative due to its easy operation and developments varieties of adsorbents. Although, many adsorbents have been utilized for Cr (VI) removal. It remains important to develop alternative more cost effective adsorbents. Since rice husk possess different characteristics following different methods of treatment by physically, chemically and organically modification improved the adsorption capacity of active binding site by either locally or in advance manner so as to find the most cost effective methods of rice husk treatments to improve its adsorption efficiency.

MATERIALS AND METHODS

Sampling

The study was conducted at Challawa Industrial Estate located in Kumbotso Local Government area Kano State. Effluent samples were collected from Mario Jose Tannery at four different points and reference water sample from well (Yandoka).

W1: Influent sample within the tanning yard.

W2: Effluent sample a short distance of 100 m away from the point of discharge

W3: Stream at a distance of 2.5 km from the Mario Jose Tannery

W4: Reference water sample well (Yandoka). A distance of 5 km away from the tannery.

The effluents were kept in an ice box at 4°C and transported to the laboratory. The samples were analyzed immediately upon reaching the laboratory.

Physical/chemical properties of tannery effluent

pH

Twenty five (25) ml of the effluent waste was pipette into 50 ml of a beaker, the effluent waste was then filtered through nylon cloth and suspended particles was then allowed to stand for one hour before using the electrode in the clamps of the electrode holder down into the beaker. The glass electrode was immersed just deep enough into the clear supernatant solution to establish a good electrical contact through the ground glass and was read on the pH (Black, 2006).

Acidity

One hundred (100) ml of the sample was introduced into a 250 ml Erlenmeyer flask with three drops of phenolphthalein indicators. The solution was then titrated with 0.02N NaOH from a burette until the first permanent pink colour appeared and recorded the ml of sodium hydroxide used. The acidity was calculated using milliliters of 0.024 NaOH $\times 10 = \text{mg/L}$ total acidity expressed as CaCO_3 (Nag, 2007).

Alkalinity

One hundred (100) ml of the sample was introduced into a 250 ml Erlenmeyer flask followed by addition of three drops of phenolphthalein indicator upon the development of a pink colour

which just disappears, and the amount of acid used was recorded. Three drops of methyl orange indicator was then added to the flask. If the sample became yellow, then 0.02N sulphuric acid was added until the difference in colour was noted when compared with the distilled water. The alkalinity was calculated using total alkalinity as CaCO_3 (mg/L) = Total ml acid $\times 10$ (Nag, 2007).

Total suspended solids (TSS)

In waste water sample (TSS) was determined through laboratory experiments of the liquid in an oven. A residue retained on the filter was dried to a constant weight at a temperature of 105°C.

$$\text{TSS (in mg/L)} = \frac{(B-A) \times 10^6}{V}$$

Where, A is the weight of filter paper, B is the weight of paper and residue, V is volume of the sample taken (Sharma, 2009).

Total dissolved solid (TDS)

Fifty ml of the waste sample was filtered through a Whatman's filter paper No1. The titrate was then transferred in a pre - weighed evaporating dish and placed in a hot water bath for evaporation. The dried solid content was placed in a desiccator to cool and re weight at regular interval of 30 min for consecutive three times until constant interval of weight was obtained. The total dissolved solids wastes were calculated using.

$$\text{TDS (in mg/L)} = \frac{(B-D) \times 10^6}{V}$$

Where, D = initial weight of the dish (g), B = Final weight of the dish (g), V = Volume of the water sample taken.

Chemical oxygen demand (COD) and biochemical oxygen demand BOD were conducted according to Nag (2007).

Total nitrogen (TN)

One and half gram of crushed dried soil was poured into 800 ml Kjeldahl flask along with 25 ml of conc. H_2SO_4 and 3 g of mixed catalyst (NaHCO_3). The sample was digested using Kjeldahl digestion apparatus until a clear green or whitish colour was obtained. The digested solution was then diluted to 100 ml with distilled water. Distillation was done by adding 20 ml of digested solution into 500 ml Kjeldahl flask containing anti-bumping chips and 40 ml of 40% NaOH was slowly added by the side of the flask. A conical flask (250 ml) containing a mixture of 50 ml of 2% boric acid and four drops of mixed indicator (Cresol/bromothymol) was used to trap the liberated ammonia. The distillate was then titrated with HCl. The total nitrogen content was then calculated using

$$\%N_2 = \frac{14 \times M \times V_t \times V \times 100}{\text{Weight of sample (mg)} \times V_s}$$

Where, M = actual molarity of acid, V = titre volume of HCl used, V_t = volume of diluted digest, V_s = aliquot volume of distilled (Onyeike and Osieji, 2003).

Total chromium (TC)

Fifty gram of the dried crushed soil was suspended in 50 ml distilled

water in a beaker and was filtered through nylon cloth. Twenty five (25) of the filtrate was collected in a 400 ml beaker and 10 ml of concentrated H_2SO_4 and 5 ml of concentrated HNO_3 were added to the filtrate. The beaker containing the mixture was then placed on a hot plate for boiling until the solution becomes clear and then the solution was transferred by filtration through Whatman Filter paper into a volumetric flask. The volume of the filtrate was made up to 50 ml by adding deionized water. Digested sample were stored in sterile polyethylene bottles at room temperature for further analysis of the metal using flame atomic absorption spectrometry (FAAS) (Rani, 2003).

Treatment of agricultural waste (saw dust and rice husk)

Agriculture waste either saw dust or rice husk was procured from Sabon Gari market, Zaria, Nigeria and brought to the Laboratory for analysis. The waste was re-grounded and sieved to obtain a fine texture. Two hundred and fifty grams of fine texture was weighed and added to 1000 ml of basal media with the following composition (g/l): NH_4SO_4 - 1.0; K_2HPO_4 - 1.0; $MgSO_4 \cdot 7H_2O$ - 1.0; $CaCO_3$ - 4.0; $NaCl$ - 1.0 and $NaNO_3$ - 6.0; K_2HPO_4 - 2.0; $MgSO_4 \cdot 7H_2O$ - 0.20. The mixture was then mixed and boiled to semi-solid slurry after which a prepared solution of 250 g of yellow purple *P. biglobosa* (dorowa) was added and boiled to breakdown the complex organic compound to simpler substances. The paste slurry was later spread on a dried sterile tray and oven dried at 40°C for seven days. The pH of the paste slurry was brought down using *T. indica* (Tsamia) from 3.5 to 4.5 and was dried for further analysis.

Biosorption studies using selected fungi

The absorptive capabilities of the three fungi species were selected after testing for their tolerance to $CrSO_4$ solution at 4% concentration in the laboratory, while the less tolerance organisms died at 1% concentration (Gbolagunte et al., 2003). Single and combination of these tolerable fungi were studies in modified culture medium viz; *Aspergillus niger*, *Rhizopus nigricans*, *Penicillium* sp and a mixed culture of all the three fungi. Twenty gram of the modified rice husk medium was added to different volumes (50, 65, 75, 85 and 100 ml) of tannery effluent in 250 ml conical flask. The pH was adjusted to 5.2 and sterilized in an autoclave at 121°C for 15 min. The solution was allowed to cool. The inoculum of fungi cell suspension was prepared by adding 5 ml of sterile distilled water to freshly grown fungal mycelia in slant agar bottle. 4 ml of spore suspension containing *A. niger* was then inoculated into the conical flask containing sterile modified rice husk medium (MRHM) and effluent. The conical flasks were incubated for 0, 15, 30 and 45 days at 32°C. These procedure was carried out using potato dextrose medium (PDM) as the growth substrate and *R. nigricans*, *Penicillium* sp. and mixed culture as inoculants. All the samples were filtered at specific interval using Whatman filter paper No 4. The filtrate or the supernatant liquid obtained after removal of mycelia mat was used for Cr (VI) ions analysis.

Determination of chromium (VI) content of remediated effluent

Principle

Solution chromium (VI) is leached from the sample at 7.5 to 8.0 under inert gas, the chromium (VI) in solution oxidizes 1,5 diphenylcarbazides to 1,5 diphenylcarbazone to give a red/violet complex with chromium which is quantified photometrically at 540 nm chemicals.

Diphenylcarbazides solution

One gram of 1,5 diphenylcarbazides CO ($NHNHC_6H_5$)₂ was

dissolved in 100 ml acetone (CH_2)₂CO and made acidic with one drop of glacial acetic CH_3COOH . This was kept in brown glass bottle. The shelf life was up to 14 days at 4°C.

Chromium (VI) stock solution

2.829 g potassium dichromate ($K_2Cr_2O_7$) dried for 16 h at 102°C was dissolved in water in a volumetric flask and made up to 1000 ml with water. 1 ml of this solution contains 1 mg of chromium.

Chromium (VI) standard solution

One (1) mg of solution was pipetted in to the mark with distilled water. 1 ml of this solution contains μg chromium.

Procedure

Ten (10) ml of this supernatant liquid was pipetted into a 50 ml volumetric flask. The solution was diluted with water; 1 ml of diphenylcarbazides solution was added followed by 1 ml phosphoric acid with distilled water, after which 10 min the pink colouration solution was analyzed for Cr (VI) ions using spectrometer at absorbance of 540 nm against the black solution. Another 10 ml aliquot of the solution was pipette into a 50 ml volumetric flask and treated as described above but without the addition of the diphenylcarbazide solution. The blank solution was prepared by filling 50 ml distilled water. Then, 1 ml of diphenylcarbazides solution and 1 ml of phosphoric acid were added to make up the mixed throughout and was stored in a dark and cool place. The calibrating solutions are prepared in 50 ml volumetric flask. A suitable calibration curves standards containing 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2.5, and 3 μg Cr were taken and acidized with 0.2N of 5 ml of 1,5 - diphenyl carbazides was added 10 ml treated sample until a pink coloured was develop and the absorbance measurement was performed at 540 nm with spectrophotometric data of calibration standards (Addis, 2006).

RESULTS AND DISCUSSION

The results of the physical characterization of the tannery effluent are presented in Table 1. The colour is usually the first contaminant to be recognized in waste waters that affect the aesthetics water transparency and gas solubility of water bodies. Such colour were observed to be dark brown (W_1 , W_2 , and W_3 except W_4) which is colourless from control sample. Dhungana and Yadav, (2009) who reported colourless, dirty dark green and green, appearance for tannery effluents of different tanneries from Bara and Parsa districts of Nepal. pH is define as the negative log of hydrogen concentration. It indicated the acidity and alkalinity of water sample. The hydrogen ion concentration is influenced by biological activities. Beside, in addition of chemical substance, presence of organic matter in large quantity may lower the pH value due to release of CO_2 and SO_2 . The pollution load in most of the chemical industries is in large quantity in the form of acid and alkali in manufacturing unit. The wide variation in the pH value of effluent can affect the rate of biological reaction and of various microorganisms. The presence or absence of various

Table 1. Physico - chemical characteristics of effluent sample from MarioJose, reference water sample from stream and Yandoka well.

Parameter	W ₁	W ₂	W ₃	W ₄
Colour	Fairly	Dark	Dark	Colourless
	Dark	Brown	Brown	
	Brown			
Odour	Foul	Foul	Foul	Odourless
	Smell	Smell	Smell	
pH	6.8	6.7	6.8	7.4
Temperature(°C)	30	31	30	31
Total chromium (mg/l)	1.577	2.478	6.624	0.07
Nitrogen (%)	0.07	0.070	0.011	0.07
COD (mg/l)	600	800	200	40
BOD (mg/l)	5.5	20.2	150	40
Acidity (mg/l)	3.00	4.50	3.40	7.20
Alkalinity (mg/l)	23	28	40	7.4
Dissolved solid (mg/l)	100	200	400	50
Total suspended solid(mg/l)	400	540	600	20

W₁ = Mario Jose Influent sample within the tannin yard, W₂ = Effluent sample at the point of discharge, W₃ = Effluent sample from stream, W₄ = Reference water sample.

ionic species can have the direct relation with pH of the effluent. Subsequently, such effluent can influence the quality of the soil. The reaction between effluent flowing from open drainage system and the soil has direct relevance to the pH of the effluent. It is therefore necessary to evaluate with respect to the pH value. Above neutral pH value was recorded (7.4) W₄. However, this value falls within the permissible limit recommended by (WHO). The discharge of waste water into water bodies may cause a drop or increase in their pH value may be due to the size and activities of microbial populations.

In this present investigation, the samples recorded the pH value nearest to neutral in the effluent waste treatment W₁ (6.8), W₂ (6.7), W₃ (6.8) whereas the pH of the control water sample W₄ was 7.4. Deepali et al. (2009), reported pH of the tannery effluents as 3 to 3.5 which was a very acidic condition. Whereas, Krishnamoorthi et al. (2009), observed pH of tannery as 8 to 9. However, WHO guide line the tolerance limit of pH 6 to 9 (Akan et al., 2008). Temperature is important for its effects on certain chemical and biological reaction taking place in water and in organism inhabiting aquatic media and will depend upon season and time of sampling. No specific limit for temperature is prescribed by WHO or ISI for the water quality use for the domestic purpose.

In the present investigation, the temperature values observed were W₁ (30), W₂ (31), W₃ (30) and W₄ (31) respectively. Measurement of temperature is an important parameter required to get an idea of self purification

capacity of river, reservoir and control of treatment plant. Water temperature is also important parameter for aquatic life. It is an important factor for calculating solubility of oxygen, carbon dioxide, bicarbonate, and carbonates. Temperature of drinking water has an influence on its taste. During the summer, water temperature is usually higher because of decrease in water table, clear atmosphere and great solar radiation, while in rainy season can be explained on the basis of cloudy atmosphere, high percentage of humidity and high water table. High temperature could be attributable to the addition of warm water from industrial activities. Increase in temperature can cause change in the species of fish that is existing in the receiving body.

The values were higher than the WHO (2003) standards. The results indicate that some reactions could be speeded up by the discharge of this waste water into stream. It will also reduce solubility of oxygen and amplified odour due to anaerobic reaction (Akan et al., 2008).

The lowest total chromium (0.07 mg/l) was found in W₄ followed by (1.577 mg/l) W₁ and the highest in W₃ (6.624 mg/l). The result is in line with study conducted by Akan et al. (2005). However, the permissible limit for total chromium discharge in the stream or river for irrigation and domestic used should not exceed 0.05 mg/l by WHO. A possible explanation for its high level is as result of the used chromium salt during tanning. This could be disastrous to the concept of a clean environment. It may also enter the food chain through plants, animals as well

as water source. Once it gets into food chains by biomagnifications and bioaccumulation of the metal in various living systems may take place. This result is in conformity with that of (Khan, 2006), in which they reported that bioaccumulation and biomagnifications could lead to toxic level of these metals in organism even, if exposure level is low. This could also cause disruption in the ecological balance when in abundance. However, the said permissible limit for total chromium discharge in the stream or river for irrigation and domestic used should not exceed 0.05 mg/l by (WHO, 1985). Then, it could be that the rural dwellers that leave within that vicinity are not guarantee of safety. High concentrations of chromium in drinking water can cause skin ulcer, allergic reactions, carcinogenic and mutagenic effect to humans (Martin and Ginswold, 2009). The lowest nitrogen concentration was found in W_1 (0.07%) followed by W_3 (0.011%) and in comparison with the control sample W_4 (0.07%). The present findings markedly agreed with study of Narasimba (2009). Thus, the presence of nitrogen compounds added with some organic debris from hides and skin of animals have attributed to their trace (Gbolagute et al., 2003).

Biochemical oxygen demand (BOD) is defined as amount of oxygen required by microorganisms, while stabilizing biological decomposable organic matter in a waste under aerobic conditions. Since the test is mainly a bioassay procedure, involving measurement of oxygen consumed by bacteria while stabilizing organic matter under the aerobic conditions. It is necessary to provide standard conditions of nutrient supply and pH. Absence of microorganisms due to the low solubility of oxygen in water and strong wastes are always diluted to ensure that the demand does not increase in available oxygen. Low value of BOD is comparatively in winter months may be due to lesser quantity of total solids, suspended solids in water as well as to the quantitative number of microbial population (Avasan and Rao, 2001). In this current investigation, the highest BOD was recorded in W_3 (150 mg/l) and in comparison with the BOD of the reference water sample W_4 (40 mg/l). It has been reported by Nouri Sepher et al. (2005), that the range of BOD in tannery waste water is 3000 to 3500 mg/l. The greater decomposable matter present the greater the oxygen demand and the greater the BOD values (Ademoroti, 1996).

The chemical oxygen demand test (COD) determines, the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. The COD is a test which is used to measure pollution of domestic and industrial waste. The waste is measure in terms of organic matter to produce CO_2 and H_2O . It is a fact that all organic compounds with a few exceptions can be oxidizing agents under the acidic condition and presence of biological resistant substances. In this present study there was remarkable decrease in concentrations of COD at different stages of effluent waste treated. The highest

was recorded at W_2 (500 mg/l) followed by W_1 (600 mg/l) and W_3 (21 mg/l) and in comparison with the COD of the reference water sample W_4 (40 mg/l). Trivedi and Goel (1986) observed COD value of textile industry ranges from 300 to 2400 mg/l. Total dissolved solid (TDS) is the total ion concentration of total dissolved solid in summer which increased in rainy seasons while the minimum value was found in winter notably because of stagnation. In summer most of the vegetation is decaying, so, rise in the amount of dissolved solids was neutral as the products of decaying matter which were settled in the water. The total solid concentration in waste effluent represents in the colloidal form and dissolved species. The probable season for the fluctuation of value of total solids and subsequent the value of dissolved solids is due to content collision of these aggregated process is also influence by pH of these effluents. In the rainy season less concentration of total dissolved solid is obtained due to the concentration of the dissolved solids and also due to the dilution of waste effluent with rainy water.

In the present study, the results of total dissolved solid of the maximum concentration recorded W_2 (400 mg/l), followed by W_2 (200 mg/l) and W_1 (100 mg/l) and in comparison with the TDS of reference water sample W_4 (50 mg/l). Hossetti et al. (1994) reported that total dissolve solid in range 488 mg/l in the waste water from Jayanthi Na'illa. Total suspended solid (TSS) does not mean that they are floating matters and remain on top of water layer. They are under suspension and remain in water sample. This has not set any limit of total suspended solids for drinking water. Total suspended solids play an important role in water and waste water treatment. Their presence in water sample causes depletion of oxygen level Devi (1980) reported total plankton which showed a streaking parallel are form with suspended solids. Effluents from different industries may have the different amount of solids particulate matter when the effluent flows through the open drainage system particulate which is expected to show greater degree of variance. If the effluent is highly acidic then the solid may dissolved on it, therefore it is necessary to evaluate effluent for the particulate matter. In the present study suspended solid content of the different stages of effluent waste. The highest value was recorded W_3 (600 mg/l) followed by W_2 (540 mg/l) and W_1 (400 mg/l) which in comparison with TSS of the reference water sample W_4 (20 mg/l). Avassan and Rao (2001), observed the TSS and sugar mill effluent as 220 to 790 mg/l.

Three fungi were selected for biosorption studies using agricultural wastes medium, that is, modified rice husk medium (MRHM). The three fungi were viz; *A. niger*, *R. nigricans*, *Penicillium* sp and mixed culture organisms. High rate of absorption was considerably observed by *R. nigricans* of the three fungi on (MRHM) which showed 85% reduction in Cr(VI) ion ranging from initial concentration of (1.517 to 0.067 mg/l) after 45 days

Table 2. Chromium (VI) biosorption of effluent sample from Mario Jose tannery by selected fungal isolates grown on different media.

Fungal isolate	PDM				MRHM			
	Initial conc.	15 days	30 days	45 days	Initial conc.	15 days	30 days	45 days
Location (Mario Jose tannery)								
<i>Aspergillus niger</i>	1.517	1.201	1.301	1.301	1.517	1.208	0.210	0.072
<i>Rhizopus</i>	1.517	1.012	1.013	1.013	1.517	1.302	0.325	0.067
<i>nigricans penicillium</i> sp.	1.517	1.412	1.328	1.329	1.517	1.249	0.430	0.073
Mixed culture	1.517	1.303	1.287	1.384	1.517	1.309	0.241	0.074

PDM, Potatoes dextrose medium; MRHM, modified rice husk medium. Values are in mg/l.

Table 3. Chi square test potato dextrose medium (PDM) submerged fermentation.

Fungal isolates	Days			
	15 days	30 days	45 days	Total
<i>Aspergillus niger</i>	1.201	1.301	1.301	3.803
<i>Rhizopus nigricans</i>	1.012	1.013	1.013	3.038
<i>Penicillium</i> sp	1.031	1.412	1.328	4.257
Mixed culture	1.303	1.303	1.287	3.893
Total	5.303	5.029	4.929	14.991

Testing of hypothesis, $\alpha = 0.05$, Critical value with (3-1) (3-1) = (2) (2) – 4, Degrees of freedom at $\alpha = 0.05 = 9.488$, Chi - square test value = 0.0157647, Decision - Do not reject null hypothesis since 0.015 < 9.488

observed by mixed culture. *Penicillium* sp. and *A. niger* of the three fungi growing on PDM where a reduction of 9% was achieved from an initial concentration of 1.517 to 1.013 mg/l of tannery effluent from Mario Jose tannery after 45 days incubation (Table 2). These findings agree with the study conducted by Ugoji and Aboaba (2004). Chromium ion in polluted land which had higher concentration 89.30% against the lower value of 0.25 mg/l in the control. Biosorption largely involves physical and adsorption following chemical bondage and does not required energy. Once the metal ions are diffused on the cells surface, they bind to site, which exhibit chemical affinity for the metal. It is a passive accumulation process which may include adsorption - exchange complexation, chelation and micro precipitation. Chromium resistant fungi have been isolated from tannery effluent by several groups (Sultans and Hasains, 2007). Such physiological studies includes the following (carboxylic, hydroxyl and phosphate group of lipids proteins polysaccharides), localized at the cell surface for maximum adsorption (Preetha and Viruthagiri, 2005).

In this present investigation, the possible mechanism of hexavalent chromium reduction by two fungi isolated from tannery waste by biosorption studies has been evaluated. *R. nigricans* and *A. niger* showed excellent ability to reduce hexavalent chromium ranging from initial concentration 1.517 to 0.067 mg/l. Since, both the isolates have been identified as potential microbes for

their usefulness in removing chromium from the tannery effluent. The technology when up graded will be a boost to tannery in tackling the pollution problems of tannery waste water. The processes would not only be economical but also eco friendly and sustainable. Using Chi - square test analysis for fitness (PDM) as shown in Table 3, the microbial activities are effective across the days, showing no reduction in the concentration of chromium (VI) ions. The biosorption of chromium by fungi is highly maintained at a considerable level at 15 days owing to the depletion of substrate by the fungi with a higher value of Fcal 0.015 using the Chi - square analysis for fitness test as shown in Table 4. This shows that the concentration was higher at 15 days initially effective biosorption activities of microbial organisms subsequently 30 and 45 days shows a considerable decrease in effectiveness in microbial activities due to the reduction of concentration of chromium (VI) ion Fcal 0.102.

For continued microbial activities to be fully achieved, the concentration of MRHM should be kept in a constant supply of nutrient by open batch culture. For the effective bioremediation process to be achieved in Mario Jose tannery effluent MRHM by solid state fermentation should be recommended than the submerge fermentation (PDM) which involved a lot of process. Pictures were also taken in different Plates 1, 2, 3, 4 and 5 to highlight the nature of the research work being done *in-situ*.

Table 4. Chi square test modified rice husk medium (MRHM) solid state fermentation.

Fungal isolate	Days			Total
	15 days	30 days	45 days	
<i>Aspergillus niger</i>	1.208	0.210	0.072	1.490
<i>Rhizopus nigricans</i>	1.302	0.325	0.067	1.694
<i>Penicillium</i> sp	1.249	0.430	0.073	1.752
Mixed culture	1.309	0.241	0.0742	1.624
Total	5.068	1.206	0.286	6.54

A = 0.05, Critical value with (3-1) (3-1) = (2) (2) = 4, Degrees of freedom at $\alpha = 0.05 = 9.488$, Chi - square test value = 0.01257647, Decision - Do not reject null hypothesis since $0.015 < 9.488$.

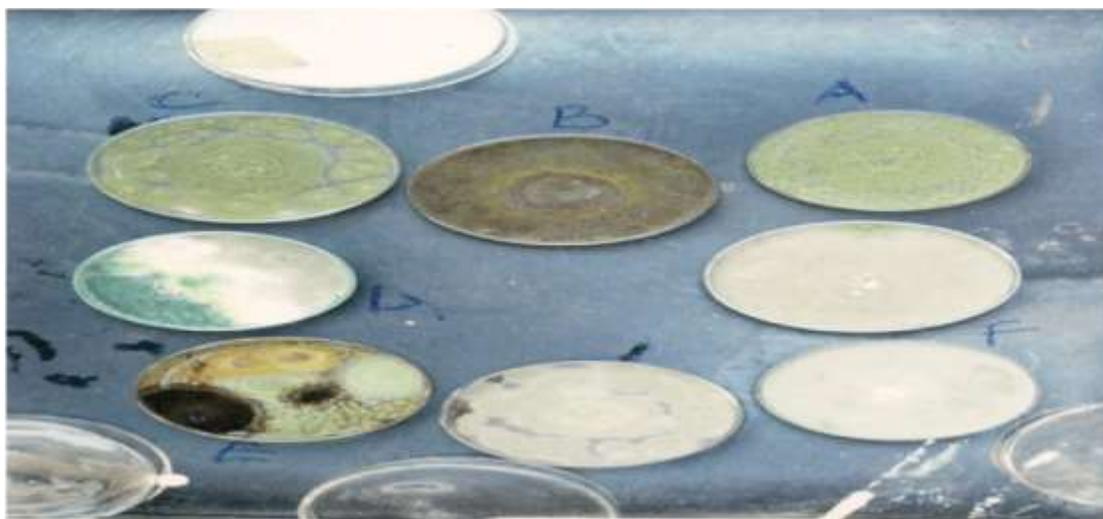


Plate 1. Mixed culture from tannery effluents /chrome buffing dust. A- *Aspergillus fumigatus*, B- Old culture of *Aspergillus niger*, C-*Aspergillus fumigatus*, D- *Penicillium* sp, E- *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*



Plate 2. *Rhizopus nigricans* from tannery effluents/chrome buffing dust.

Conclusion

Hexavalent chromium is highly toxic-pollutant introduced to natural water due to discharge of industrial waste water. Waste water from tanneries operation is one of the main sources to Cr(VI) contamination. Daily wayer residing in the premises of Challawa industrial estate of Kumbosto local government Kano state have fallen prey to Cr(VI) contamination of their water bodies that has affected their health drastically. It is our primary objective to decontaminate the water in this area in order to provide a means to obtain safe drinking water to the dwellers.

Conflict of interests

The authors have not declared any conflict of interests.



Plate 3. Modified rice husk (MRHM).

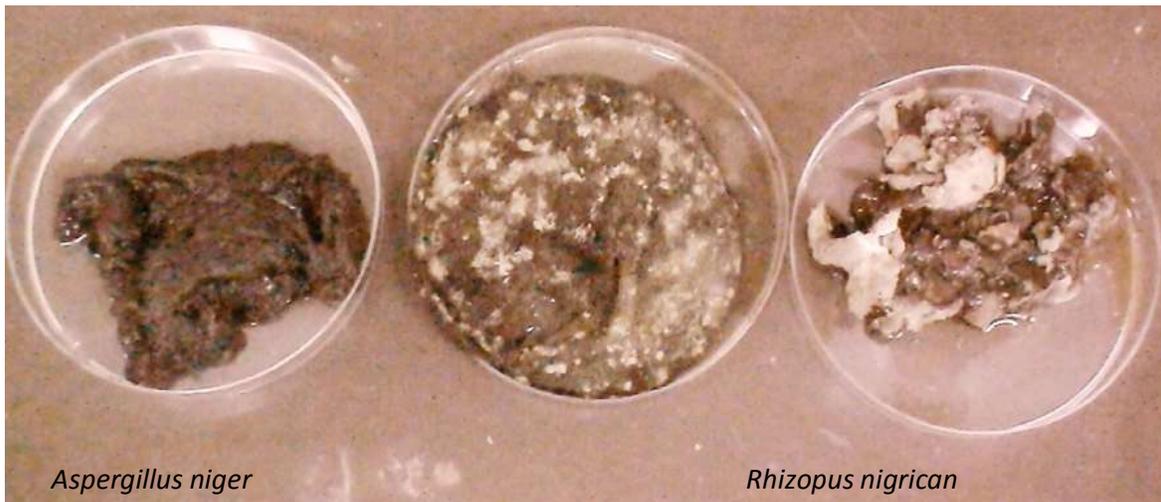


Plate 4. Fungal by-product (biomass) of *Aspergillus niger* and *Rhizopus nigricans* after 45 days incubation.



Plate 5. *Penicillium* sp by-product (biomass) after 45 days incubation.

ACKNOWLEDGEMENTS

The authors wish to thank the Director General/CEO of Nigerian Institute of Leather and Science Technology (NILEST) Samaru- Zaria, Nigeria for financial support for this work.

REFERENCES

- Addis B (2006). Determination of chromium (III) and chromium (VI) in the tannery effluent of a wash and Addis Abba leather industrial M.S.C project submitted to department of chemistry. Addis Abba University School of Graduate Studies. 25-31.
- Ademoroti CMA (1996). Standard method for water and Effluents Analysis. Foludex press Ltd, Ibadan pp. 22-23, 44-54, 111-112.
- Akan JC, Moses EA, Ogunbuaja VO (2005). Determination of pollutant level in Mario Jose Tannery Effluent from Kano Metropolis, Nigeria. Niger. J. Appl Sci. 7(4):527-530.

- Akan JC, Abdulrahman FI, Dimari GA, Ogugbuaja VO (2008). Physicochemical determination of pollutants in waste water and vegetable samples along the Jakara waste water channel in KanoMetropolis, Kano State, Nigeria. *Eur. J. Sci. Res.* 28:122-23.
- Avasal NY, Roa SR (2001). Effect of sugar mill effluent on organic resources of fish. *Poll. Res.* 20(2):167-171.
- Babu BV, Gupta S (2008). Adsorption of Cr (VI) using activated neem leaves kinetic studies. *Adsorption* 14:85-92.
- Bansal M, Garg U, Singh D, Garg VK (2009). Removal of Cr (VI) from aqueous solutions using pre-consumer processing agricultural waste: A case study of rice husk. *J. Hazard. Mater.* 162:312-320.
- Black H (2006). Absorbing possibility phytoremediation. *J. Environ. Health Perspect.* 103:1106-1108
- Deepali KK, Gangwar R, Joshi BD (2009). Comparative study of physico-chemical properties of effluent from tannery industries Indian *J. Environ Sci.* 3(2):49-50.
- Devi A (1980). Ecological studies of limon plankton of three fresh water body, Hyderabad Ph.D. Thesis Osmania University, Hyderabad.
- Dhungana TP, Yadav PN (2009). Determination of chromium in Tannery Effluent and Study of Adsorption of Cr (VI) on Sawdust and Charcoal from Sugacane Bagasses. *J. Nepal Chem. Soc.* 23:93-101.
- El-Sherif IY, Ashmawy A, Badr S (2008). Biosorption of cadmium and nickel by Nile algae. *J. Appl. Sci. Res.* 4(4):391-396.
- Espinola A, Adamian R, Gomes LMB (1999). An innovative technology: Natural coconut fibre as adsorptive medium in industrial wastewater cleanup. *Waste Treat. Clean Technol. Proc.* 3:2057-2066.
- Foo KY, Hameed BH (2009). Utilization of rice husk ash as novel adsorbent: A judicious recycling of the colloidal agricultural waste. *Adv. Colloid Interface Sci.* 152(1-2):39-47.
- Gbolagunte GO, Okonko EM, Okizie NO, Silas DE (2003). Biotechnological detoxification of chrome from tannery waste. Preliminary studies. *Nig. J. Biotechnol.* 12 (1): 9 - 17.
- Gonzalez CF, Ackerley DF, Park CH, Martin A (2003). A soluble flavoprotein contributes to chrome reduction and tolerance by *Pseudomonas putida*. *Acta Biotechnol.* 23: 233-239.
- Hosetti BB, Kulkarni AR, Patil HS (1994). Water quality in Vayanthi, Nala and Panchganga at Kolhapur. *Indian J. Environ. Health* 36(2): 124-127.
- Janick KJ (2008). *Parkia biglobosa* African Locust Bean. The encyclopedia of fruit and Nuts. Willingford U.K CABI North American office 395-400.
- Kapoor A, Viraraghvan T, Roy Cullimore D (1995). Removal of heavy metals using *Aspergillus niger*. *Bioresour. Technol.* 70: 95-104
- Khan AG (2006). Relationship between Cr. biomagnification ratio, accumulation factor and mycorrhizae in plants growing on Tannery effluent-polluted soil. *Environ Int.* 26:417-423.
- Krishanamoorthi S, Sivakumar V, Saravanan K, Prabhu S (2009). Treatment and Reused of Tannery Waste Water by embedded System. *Mod. Appl. Sci.* 3(1):129-134.
- Kumar S, Upadhyay SN, Upadhyay YD (1998). Removal of phenols by adsorption on fly ash. *J. Chem Technol. Biotechnol* 37(4): 281-287.
- Lin JL, Keener HM, Essenhigh RH (1995). Pyrolysis and combustion of corn cobs in a fluidized bed: Measurement and analysis of behavior. *Combust Flame* 100:271-282.
- Martin S, Grisworld W (2009). Human Health effluent of heavy metals center for hazardous substance research, Kansas State University, issue 15. <http://www.enqq.ksu.edu/CHS4> assessed 23:12
- Nag A (2007). Analytical techniques in Agriculture Biotechnology and Environment Engineering, prentice - hall of India New Delhi 84 -102.
- Narasimha G (2009). Physico-chemical and Biological properties samples collected from soil contaminated with effluent of cotton ginning industry. *J. Environ. Biotechnol.* 20:235-329.
- Nouri Sepehr M, Nasseris S, Mazaheri AM, Yaghmaian K (2005). Chromium Bioremoval from Tannery Industries Effluent by *Aspergillus oryzae*. *Iran J. Environ. Health Sci. Eng.* 2(4): 273-279.
- Ntui VO, Uyuh EA, Urua IS, Ogbuv, Okpako EC (2012). Regeneration of *Parkia biglobosa* Benth: An important tree species of Africa. *J. Microbiol. Biotechnol. Res.* 2(1): 167-177.
- Olaniji A (2013). Locust Bean Products Non- wood News No10 Retrieved <http://www.Fao.org/docrep/005/y464e/y464e02.htm#paia1309276>.
- Onyeike EN, Osuji JO (2003): Research Techniques in Biological and Chemical Science. Springfield Publishers Ltd, Owerri.
- Pandey PK, Verma Y, Choubey S, Pandey M, Chandrasekhar K (2008). Biosorptive removal cadmium from contaminated groundwater and industrial effluents. *Bioresour. Technol.* 99:4420-4427.
- Preetha B, Viruthagiri T (2005). Biosorption of zinc (II) by *Rhizopus arrhizus*: equilibrium and kinetic modeling. *Afr. J. Biotechnol.* 4(6): 506-508.
- Rani F (2003). Role of microorganisms in bioremediation of heavy metal ions and organic pollutants present in textile industry. Unpublished Ph.D thesis submitted to the Department of Biological Sciences, Quaid-I-Azam University, Islamabad Pakistan.
- Saeed A, Iqbal M (2003). Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arietinum*). *Water Res.* 37:3472-3480.
- Saeed A, Iqbal M, Akhtar MW (2005). Removal and recovery of heavy metals from aqueous solution using papaya wood as a new biosorbent. *Sep. Purif. Technol.* 45(1): 25-31
- Sharma KB (2009). Biodegradation of Paddy Staw obtained from different geographical locations by means of *Philebia* sp. for animal feed. *Biodegradation* 22:143-152.
- Sultans D, Hasains S (2007). Reduction of toxic bivalent chromium by *Chrobacterium*. *Int. Medium Technol.* 98:340-344
- Tarley CRT, Arruda MAZ (2004). Biosorption of heavy metals using rice milling by-products: Characterisation and application for the removal of metals from aqueous effluents. *Chemosphere* 987-995.
- Tewaria N, Vasudevana P, Guha BK (2005). Study on biosorption of Cr(VI) by *Mucor hiemalis*. *Biochem. Eng. J.* 23:185-192.
- Thiombiano DN, Lamien N, Dibong DS, Boussim IJ, Belen B (2012). The role of woody species in managing food shortage in Burkina Faso sechrasse 23(2): 86-93.
- Trivedi RK, Goel PK (1986). Characterisation, treatment and disposal of waste water in a textile industry. *Ind. Poll. Cont.* 2(1): 1-12.
- Ugoji EO, Abaoba B (2004). Biological treatments of textile industrial effluent in Lagos Metropolis *Nig. J. Environ. Biol.* 25 (4): 497 - 02
- Wang J, Chen C (2009). Biosorbents for heavy metals removal and their future. *Biotechnol. Adv.* 27: 195-226.
- WHO (2003). The World Health organization 1211 Geneva 27, Switzerland.
- WHO, (1985). Health hazards from nitrate in drinking water. WHO Regional Office for Europe.
- Yan GC, Viraraghvan J (2003). Heavy metal removal from aqueous solution by fungi *Mucor rouxii*. *Water Res.* 27:4886-4497.