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Assessment of the Potentials of Autochthonous Lipase-Producing Bacteria in the Treatment of Palm Oil Mill Effluent

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ABSTRACT
Pollution caused by the release of untreated palm oil mill effluent (POME) is a major environmental concern. This research assessed the potentials of single and consortium of autochthonous lipase-producing bacteria as a non-invasive procedure in the biotreatment of POME. The identity of lipase-producing bacteria in POME were determined using morphological and biochemical techniques. Identified isolates were monitored for POME utilization by measurement of optical density at 600 nm in 25 ml Bushnell Haas broth supplemented with 0.25 ml sterilized POME. Individual isolates and consortium were then used for the bioremediation of POME. Quality parameters including pH, total suspended solid (TSS), oil and grease, chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), total organic matter (TOM) and total nitrogen (TN) of the raw and treated effluents were evaluated. The population (CFU/ml) of total heterotrophic, POME - utilizing and lipolytic bacteria were 3.9 x 10^6, 2.8 x 10^6 and 3.5 x 10^5 respectively. The lipolytic bacterial isolates include *Brenneria nigrifluens*, *Bacillus circulans* and *Paenibacillus pectinilyticus*. Results revealed varying pH of 5.03 and 6.81 - 7.26 for untreated and treated respectively. The lipolytic bacteria from POME caused reduction efficiency of 100% in TSS and oil and grease contents of the treated effluents relative to the raw samples. The COD, BOD, TOC, TOM and TN reduction ranged 17.90 -93.55, 1.66 - 90.52, 16.34-59.79 %, 6.18 - 80.78 % and 11.71-93.66 % respectively**.** This research indicated that POME indigenous lipase-producing bacteria improved POME quality and therefore suggestive of their potentials in the bioremediation of POME contaminated environment with the consortium being most effective.

Keywords*:* Autochthonous; Bioremediation; Lipase-producing bacteria; Microbial consortium; Palm Oil Mill Effluent

INTRODUCTION

Palm oil is an important vegetable oil derived from the fruit of oil palm (*Elaeis guinensis*) by extraction process. *Elaeis guinensis* is a perennial oil producing plant bearing several fresh fruit bunches per year. There are several stages involved in the extraction, clarification and purification of palm oil from fresh fruit bunches with different kinds of waste produced at every stage (Akhbari *et al*., 2020). The production of palm oil usually results in the generation of large quantities of wastewater commonly referred to as palm oil mill effluent (POME). That is, palm oil mill effluent (POME) is a large volume of wastewater generated from palm oil milling activities (Bala *et al*., 2014; Ganapathy *et al*., 2019). POME is a brown slurry and contains strong pollution indicators such as high concentration of organic nitrogen, suspended solids, total dissolved solids and unrecovered oil and grease, chemical oxygen demand, and biochemical oxygen demand (Kamyab *et al*., 2018; Ganapathy *et al*., 2019; Okereke and Ginikanwa, 2020).

The quality of the raw material and the production processes have significant influence on the characteristics of POME (Aliyu, 2012). The other categories of waste generated during palm oil production include solid wastes (empty fruit bunch, palm press fibre, chaff, and palm kernel shell), and gaseous emissions (from farm operations in a mechanized farm, agrochemicals and during boiling and digesting) (Kamyab *et al*., 2018).

POME is a pollutant with significant negative consequences on the receiving soil and water environments (Chan *et al*., 2010; Obibuzor *et al*., 2012). The mismanagement and indiscriminate discharge of wastewater leads to economic, social and environmental problems (Monroy, 2013; De la Peña, 2013). The release of untreated POME into the soil tampers with pH, with attendant effects on other soil physicochemical properties and consequently nutrient availability to plants (Okereke and Ginikanwa, 2020). POME also changes the soil appearance and properties in terms of vegetation, odour, colour and constitution, making the soil to lose its vegetative cover and reduces crop yields and consequently lead to water logging of the pores and subsequent death of vegetation, leaving the environment uncovered and prone to erosion (Okereke and Ginikanwa, 2020). POME can also be a major source of water body (rivers, streams and lakes) pollution either through direct effluent discharge or indirectly through leaching and runoff resulting in water body acidification and eutrophication, with the water turning brown, smelly and slimy, threatens aquatic life, jeopardizes human and environmental health and local people are consequently denied the availability of water for domestic, bathing and fishing purposes. The environmental challenges imposed by uncontrolled discharge of effluents are enormous and therefore continued to drive the interest of environmental researchers to investigate new approaches for the effective management of POME. Thus, the ultimate objective of effluent treatment is to reduce the amount of the potentially toxic compounds in POME to their prescribed acceptable threshold limit values (TLV) as set by some standards (Khan and Ali, 2018). The BOD, TSS, O and G and pH of 100 mg/l, 400 mg/l, 50 mg/l and 5 – 9 respectively are considered as discharge limits of POME (Mohammad *et al*., 2021). Several physicochemical techniques have been applied to ameliorate the polluting effects of POME but these techniques have not produced acceptable outcomes due to the negative impacts of such methods ranging from cost ineffectiveness to creating other pollutants into the environment and destruction of ecosystem values. Biological treatment has been adjudged to be a promising technique among all the methods for treating wastewater because of its relatively low handling costs, eco-friendliness, non-invasiveness and causing no additional pollution (Sahal *et al*., 2023). These biological techniques depend on the action of microorganisms in a process called bioremediation to detoxify and specifically breakdown the oil effluent (Ganapathy *et al*., 2019). These microorganisms attack contaminants enzymatically and transform them into harmless or less toxic compounds such as carbon dioxide, water and simpler compounds that do not affect the environment while deriving energy and biomass. Several types of microorganisms including bacteria, fungi, algae, and plants have been reported as viable biological agents for the bioremediation of contaminants including wastewater (Anyanwu *et al*., 2021; Alazaiza *et al*., 2022). However, Dhouib *et al.* (2006) reported that bacteria, and fungi are the microorganisms that can perform complete degradation of oil-based wastewater**.**

Biological degradation can be achieved by using microbial species that can be cultured and isolated from soils or other carrier materials (Boboye *et al*., 2023). Single species and consortium of microorganisms have been employed in the treatment of palm oil mill effluent and other oil processing wastes. The efficiency of single species to metabolize all the polluting components to acceptable threshold is limited because of differences in the characteristics of most effluents (Asses, 2009) and can only metabolize a limited range of substrates but a mixed microbial population may be more efficient for the treatment or remediation due to their broad enzymatic capacities (Britton, 1984).

Ganapathy *et al.* (2019) reported that the treatment of oil effluents specifically involves different microbes that produce lipase enzyme in the oil effluent. Lipases (Triacyclglycerol acylhydrolases EC3.1.1.3) are extracellular enzymes (hydrolases) produced by various plants, animals and microorganisms (Patel and Parikh, 2022) that catalyze the hydrolysis of fats into fatty acids and glycerol at the water-lipid interface. Microorganisms are the most frequently used source of the enzyme in biotechnological applications (Singh *et al*., 2019). Among the bacteria, *Bacillus* sp. including *B. alcalophilus*, *B. coagulans*, *B. licheniformis*, *B. subtilis*, *B. pumilus*, and *B. stearothermophilus* sp. are the best promising candidates for lipase production with *B. stearothermophilus* the most common lipase producers (Singh *et al*., 2019). Azarnia *et al*. (2006) also reported *Acinetobacter radioresistens*, *Pseudomonas aeruginosa*, *Serratia rubidaea* and *Staphylococcus caseolyticus* as potent lipase producers. Bacterial strains such as *Pseudomonas alcaligenes, P. aeruginosa*, *P. fragi*, *P. fluorescens* BJ-10, *Bacillus subtilis, B. nealsonii* S2MT produce lipases in higher quantities (Sckoczinski *et al*., 2017; Phulpoto *et al*., 2020). The overall objective of this research is to assess the potentials of autochthonous lipase-producing bacteria (single and consortium) in the treatment of palm oil mill effluent in order to ameliorate its consequences on the ecosystem.

MATERIALS AND METHODS

Sample collection

Freshly discharged palm oil mill effluent (POME) sample was collected at 10:00 am in the month of April from a small-scale palm oil mill industry located near the stadium in, Ayeka - Okitipupa, Ondo State, Nigeria. Samples for physicochemical analysis were put into one (1) liter plastic container while the samples for microbiological analysis were taken in sterile sample bottle and transported in ice chest to the laboratory for analyses within twenty - four (24) hours of collection.

Microbiological analysis of the effluent sample

Preparation of culture media and test solutions

Culture media including nutrient agar and test solutions were prepared according to manufacturer’s specification in a conical flask. Bushnell Hass medium (BHM) was composed as magnesium sulphate (0.2 g/l), calcium chloride anhydrous (0.02 g/l), potassium dihydrogen phosphate (1.00 g/l), dipotassium hydrogen phosphate (1.00 g/l), ammonium nitrate (1.00 g/l), ferric chloride (0.05 g/l) in 1000 ml of water having a final pH of 7.0 ± 0.2. The BHM was supplemented with 1 % (v/v) of the effluent to serve as carbon source. Tributyrin agar was composed of peptone (5.0 g/l), yeast extract (3.0 g/ l), agar (15.0 g/l) and 10 ml tributyrin and pH adjusted to 7.5 (Mobarak - Qamsari *et al*., 2011). All media were covered with cotton wool and wrapped with aluminum foil, the content was stirred with a magnetic stirrer and then heated to dissolve properly.

Twenty milliliter (20 ml) of each prepared medium was measured and poured into McCartney bottles, autoclaved at 121°C for 15 minutes holding time and then allowed to cool to 44 °C (Ikuesan *et al*., 2022).

Enumeration of bacterial population

Serial dilution of the effluent was prepared in a set of test tubes each containing 9 ml sterile distilled water. One milliliter **(**1 ml) of effluent sample was taken into 9 ml sterilized water in a test tube to form a stock solution, mixed thoroughly to ensure dislodgement and even distribution and transferred (1 ml) serially until the 6th dilution. Exactly 1.0 ml of dilutions 10^{-3} to 10^{-6} was transferred into sterile Petri dishes and overlaid with previously sterilized NA, BHM and Tributyrin agar and then incubated at 37 °C for 48 hrs for the growth and enumeration of total heterotrophic, POME utilizing and lipase-producing bacteria respectively. Lipolytic bacteria on the tributyrin agar produced colonies which are surrounded by clear zones (lipid hydrolysis) in the otherwise turbid culture medium. Triplicate culture plates with 30-300 colonies were selected and counted using the colony counter (Ikuesan and Fajolu, 2022). The average count was then multiplied by the dilution factor and expressed as colony forming unit per milliliter (CFU/ml) of sample.

Purification and identification of lipase-producing bacteria

Colonies of four (4) best lipase producers based on the diameter of zone of clearance on the tributyrin agar (Patel and Parikh, 2022) were selected and sub -cultured first unto fresh tributyrin agar and later nutrient agar to purify further. Purified microbial colonies were then identified using cultural, morphological and biochemical characteristics. Biochemical tests carried out include; catalase test, citrate test, hydrogen sulphide (H_2S) production, indole, urease, methyl red, VP test, motility, starch hydrolysis and sugar fermentation following the procedures described by Cheesbrough (2006). The identities of isolates were then determined using the Advanced Bacterial Identification Software (Sorescu and Stoica, 2021)

Preparation of POME, bacterial isolates and consortium.

Palm Oil Mill Effluent (POME) collected in 1 L measuring cylinder was shaken vigorously for 30 sec and allowed to stand on the bench for 1 hr to allow for sedimentation of solids and then decanted. The effluent was thereafter centrifuged at 4,000 g for 15 min for total elimination of solids (Nwuche *et al*., 2014). The resulting supernatant was sterilized at 121 ^{O}C for 15 mins and then used for the experiments. Broth culture of individual lipolytic bacterial isolates for POME treatment was propagated in freshly prepared nutrient broth (Lab M) by inoculation of 1 loopful of each strain into 100 ml sterile broth medium and then incubated at 30^{O} C (Nwuche *et al*., 2014). Lipase- producing bacterial consortium was prepared according to the method of Ghazali *et al.* (2004) and Sathishkumat *et al*. (2008).

Evaluation of POME utilization potential of the lipolytic bacterial isolates

Four best lipase producers selected based on the maximum zone of clearance and their consortium were investigated for their potential to use POME as sole source of carbon and energy by measurement of microbial growth by optical density (OD) using the spectrophotometry method. Mineral salt medium (Bushnell-Hass) was dispensed into culture tubes and sterilized by autoclaving at 121 °C for 15 minutes. The MSM broth were allowed to cool and then supplemented with 1% (v/v) of presterilized palm oil effluent. The pH of the medium was adjusted to 5.0 in line with pH of POME as previously determined. Each isolate (1 ml) and a consortium of all the isolates in nutrient broth was subsequently inoculated in separate tube containing the liquid medium.

Culture tubes were agitated daily to provide oxygen required by the aerobes for POME utilization (Ikuesan, 2017). Triplicate samples were incubated at 30°C ± 2 °C for 6 h and the optical density taken as day zero (0) using the spectrophotometer at 600 nm wavelength and subsequently at 48 hr interval for 12 days to determine microbial growth and POME utilization potentials. Culture tubes containing liquid MS medium and POME but without organism served as control.

Analysis of quality parameters and heavy metal contents of POME

Quality parameters of raw and treated POME including pH, electrical conductivity, total dissolved solid (TDS), total suspended solid (TSS), total solids (TS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total organic matter (TOM), total nitrogen (TN) and Oil and Grease (O and G) were determined by conventional physical and chemical analytical techniques described by APHA (2005).

Biotreatment of POME by lipolytic bacteria from effluents

The presterilized supernatant of POME earlier developed was dispensed in 100 ml as separate batches into 250 mL Erlenmeyer flasks. Individual lipolytic bacterium and consortium was separately inoculated at 1% (v/v) into the presterilized effluent and then incubated for 12 days at 30 °C ± 2 °C. The quality parameters of the treated and untreated effluent were then analyzed to determine the effect of biotreatment with lipolytic bacterial isolates. All the experiments were performed in triplicates. The efficiency for quality parameter reduction and the percentage reduction was then calculated by the following formula (Piro *et al.*, 2011).

$$\text{Reduction (\%)} = \frac{C_{raw\ POME} - C_{treated\ POME}}{C_{raw\ POME}} \times 100$$

Where $C_{raw\ POME}$ is the concentration of quality parameters of raw POME and $C_{treated\ POME}$ is the concentration of these parameters after treatment. Each set of these experiments was carried out three times.

Statistical Analysis

Statistical analysis of data obtained was carried out using Microsoft excel to obtain mean and standard deviations.

RESULTS

Population of bacterial types in palm oil mill effluent

The population of total heterotrophic bacteria (THB), POME-utilizing bacteria (PUB) and lipolytic bacteria (LPB) in the effluent are shown in Table 1 below. Results revealed higher population ($3.9 \times 10^6 \pm 2.40$ cfu/ml) of THB than PUB and LPB which were $2.8 \times 10^6 \pm 1.4$ (cfu/ml) and $3.5 \times 10^5 \pm 4.6$ (cfu/ml) constituting only 71.80% and 8.97% respectively relative to the total heterotrophic count.

Table 1: Population bacterial types in palm oil mill effluent

Bacteria type	Population (CFU/ml)	% to THB
THB	$3.9 \times 10^6 \pm 2.4$	-
PUB	$2.8 \times 10^6 \pm 1.4$	71. 80
LPB	$3.5 \times 10^5 \pm 4.6$	8. 97

Key: THB; Total Heterotrophic Bacteria, PUB; POME Utilizing Bacteria, LPB; Lipase-Producing Bacteria

Identity of lipase-producing POME-utilizing bacteria

Plates 1 (a-d) present the identity of lipase-producing bacteria from POME based on morphological and biochemical characteristics using the ABI software.

Results revealed the identity of lipase producing bacteria in the POME sample as *Brenneria nigrifluence*, *Bacillus circulans* (93.4 % similarity) *Bacillus circulans* (92.9 % similarity) and *Paenibacillus pectinilyticus*. *Brenneria nigrifluence* (LBI) is Gram negative while the other three isolates are Gram positive. The plates and table 2 show that the isolates had varying percentage similarity (86.7 - 93.4 %) and matrix integrity (97 - 100 %).

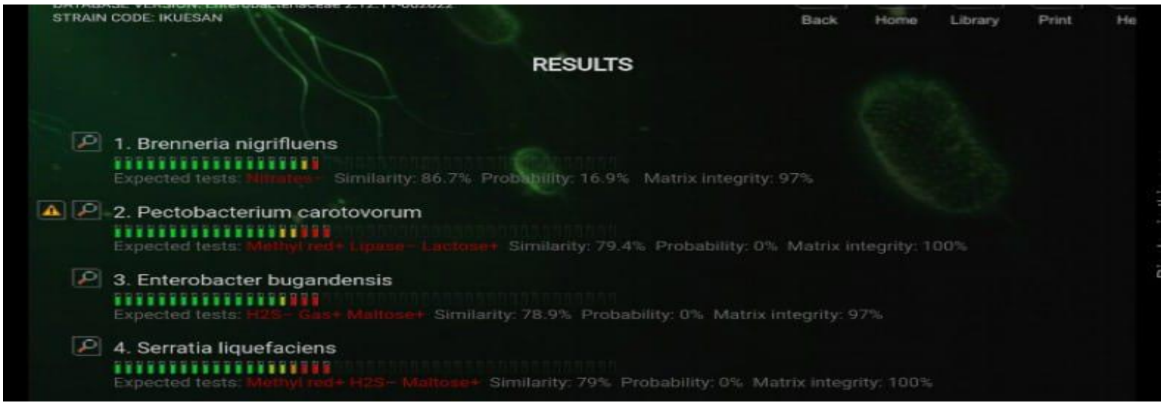

Plate 1a: Identity of lipase-producing POME-utilizing bacteria; *Brenneria nigrifluens* (LB1)

Plate 1b: Identity of lipase-producing POME-utilizing bacteria; *Bacillus circulans* (LB2)

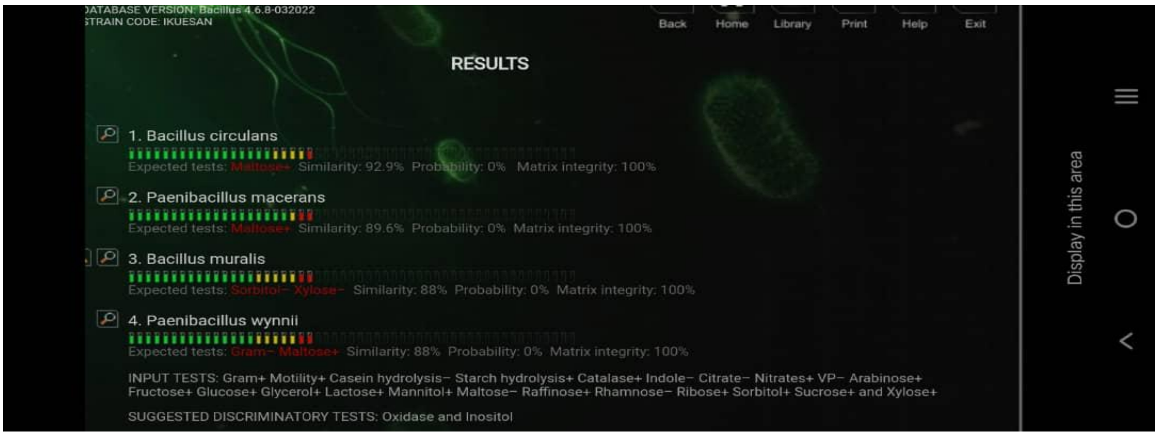

Plate 1c: Identity of lipase-producing POME-utilizing bacteria; *Bacillus circulans* (LB3)

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Plate 1d: Identity of lipase-producing POME-utilizing bacteria; *Paenibacillus pectinilyticus* (LB4)

Table 2: Identity of Autochthonous Lipase – Producing bacteria from POME.

Isolate code	Name of organism	Percentage similarity	Matrix integrity (%)
LB1	*Brenneria nigrifluens*	86.7	97
LB2	*Bacillus circulans*	93.4	100
LB3	*Bacillus circulans*	92.9	100
LB4	*Paenibacillus pectinilyticus*	91.2	100

POME utilization potentials of the isolated lipase producing bacteria

The palm oil mill effluent utilization potentials of the lipolytic bacteria isolates and consortium determined by measurement of optical density at 600 nm is shown in figure 1. The individual isolates and consortium exhibited variation in their POME utilization potentials for each of the days. LB1, LB2 and the consortium showed best potential at day 6 of growth as the cell concentration OD_{600} = 0.255, OD_{600} = 0.3035 and OD_{600} = 0.318 respectively while LB3 and LB4 were at their best of POME utilization at day 8 with cell concentration of OD_{600} = 0.2585 and OD_{600} = 0.2725 respectively.

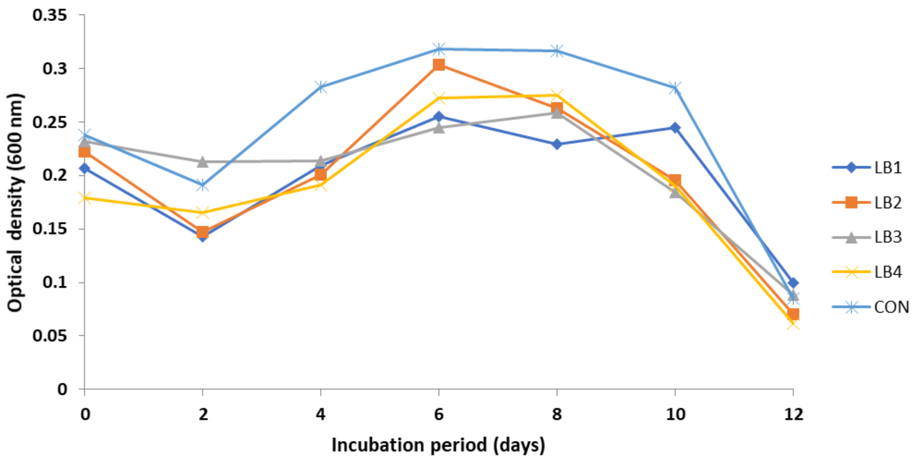

Figure 1: Effluent utilization at 600 nm by individual and consortium of autochthonous lipase- producing bacteria from POME.

Keys: LB1: *Benneria nigrifluence*, LB2; *Bacillus circulans*, LB3; *Bacillus circulans*; LB4, *Paenibaccillus pectinolyticus*, CON; Consortium of bacterial

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Effect of biotreatment on the physicochemical characteristics palm oil mill effluent

The raw POME sample used in this study was brownish in colour, having a thick and sticky appearance and oily with an offensive odour. The sample had a slightly acidic pH (5.03) Table 3. COD value of 65,100.00 mg/l. The biological oxygen demand, total nitrogen, total organic carbon, total organic matter, and oil and grease concentration of the POME were 12,050 mg/l, 1,616.5 mg/l, 3,605.1 mg/l, 9,7762 mg/l and 177.00 mg/l respectively. The sample however contain lower concentration of dissolved solid (TDS) of 1, 909 mg/l than the total suspended (TSS) of 9.092 mg/l.

Treatment of the POME with the isolated lipolytic bacteria and consortium also revealed varying degree of influence of the biological agents on all the physicochemical qualities of the effluent. Results in Table 3 show that the pH of samples treated with individual isolates ranged 6.81 – 6.92 against the 5.03 of the raw effluent while treatment with the consortium resulted in pH of 7.26.

Table 3: Physicochemical characteristics of raw and lipolytic bacteria treated palm oil mill effluent

Parameter	Raw	Treated (LB1 - *Brenneria nigrifluens*	Treated (LB2 – *Bacillus circulans*)	Treated (LB3 – *Bacillus circulans*)	Treated (LB4 - *Paenibacillus pectinilyticus)*	Treated (Microbial Consortium)
pH	5.03 ± 0.01	6.92 ± 0.03	6.89 ± 0.057	6.81 ± 0.16	6.95 ± 0.00	7.26 ± 0.21
EC (μs/cm)	321.75 ± 0.71	53.25 ± 0.71	32.35 ± 2.12	27.42 ± 0.03	18.23 ± 0.007	17.56 ±2.28
TDS (mg/ml)	1, 909 ± 0.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
TSS (mg/ml)	9.092 ± 3.62	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oil and grease (mg/l)	177 ± 2.83	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total Organic Carbon (mg/l)	3,605.1 ± 0.04	18,318 ± 0.1009	14,517 ± 0.0063	23,763 ± 7.07	30,129 ± 0.00	15,235 ± 0.00
Total Organic Matter (mg/l)	9,776.2 ± 0.06	23,021.5 ± 0.0126	19,023 ± 7.0710	81,121 ± 3.54	91,724 ± 0.01	18,792 ± 0.02
Total Nitrogen (mg/l)	1,616.5 ± 0.01	159.1 ± 0.0791	104.6 ± 0.006	1,321.8 ± 7.0711	1,427.2 ± 0.0001	102.5 ± 0.00
COD (mg/l)	65,100 ± 1.41	18,350 ± 0.71	5,350 ± 0.7	43,150 ± 0.7	53,450.5 ± 0.078	4,200 ± 1.41
BOD (mg/l)	12,050 ± 0.71	5,614 ± 0.68	2,242 ± 0.01	11,850 ± 0.7	9,750 ± 2.1	1,143 ± 0.127

Key: EC: Electrical Conductivity, TDS: Total Dissolved Solid, TSS: Total Suspended Solid, COD: Chemical Oxygen Demand, BOD: Biological Oxygen Demand.

Quality Parameter Reduction Efficiency by Autochthonous lipase producing in the bioremediation of POME.

The percentage reduction efficiency of quality parameters by autochthonous lipase - producing bacteria in the bioremediation of POME revealed in table 4 that all the isolates and consortium had 100 % reduction efficiency on the total dissolved solid, total suspended solid and the oil and grease constituents of the effluent while the reduction efficiency on the other parameters ranged 49.19-83.45 %, 59.69-93.53 %, 1.66 – 91.48 %, 6.18 -94.33 % and 57.70 – 94.54 for samples treated with *Brenneria nigrifluens* (LB1), *Bacillus circulans*(LB2), *Bacillus circulans* (LB3), *Paenibacillus pectinilyticus* (LB4) and microbial consortium respectively. Results revealed that among the individual isolates, *Bacillus circulans* (LB2) was most efficient in TOC, TOM, TN, COD and BOD removal.

Table 4: Percentage Quality Parameter Reduction Efficiency by Autochthonous lipase producing in the bioremediation of POME

Parameter (mg/l)	Raw POME Value	Percentage Quality Parameter Reduction Efficiency (%)				
		LB1- *Brenneria nigrifluens*	LB2- *Bacillus circulans*	LB3 – *Bacillus circulans*	LB4- *Paenibacillus pectinilyticus*	Microbial Consortium
*pH	5.03 ± 0.01	–	–	–	–	–
EC(µs/cm)	321.75 ± 0.71	83.45	89.96	91.48	94.33	94.54
TDS	1,909± 0.31	100	100	100	100	100
TSS	9,092 ± 3.62	100	100	100	100	100
O & G	177 ± 2.83	100	100	100	100	100
TOC	3,605.1 ± 0.04	49.19	59.69	34.02	16.34	57.70
TOM	9,776.2 ± 0.06	76.45	80.54	17.02	6.18	80.78
TN	1,616.5 ± 0.00	90.2	93.53	18.23	11.71	93.66
COD	65,100.00 ± 1.41	71.81	91.78	33.72	17.90	93.55
BOD	12,005 ± 0.71	53.41	81.39	1.66	19.09	90.52

Key: ∗Not measured in percentage, EC; Electrical conductivity, TDS; Total Dissolved Solid, TSS; Total Suspended Solid, O & G; Oil and Grease, TOC; Total Organic Carbon, TOM; Total Organic Matter, TN; Total Nitrogen, COD; Chemical Oxygen Demand, BOD; Biological Oxygen Demand

DISCUSSION

Palm oil mill effluent (POME) is a serious pollutant with significant negative consequences on soil, water, plant, animals and ecosystem health. Microbial degradation of contaminants remain the most efficient, cost effective, non-invasive and eco-friendly process of removing POME and other pollutants from the environment (Ganapathy *et al*., 2019). The present study reports the isolation and identification of autochthonous lipase- producing bacteria from POME and their potentials in the biotreatment of the effluent for improved quality before discharge into the environment. Results in this research indicated that the population of total heterotrophic bacteria were higher than total POME- utilizing and lipase- producing bacteria which were 71.80 % and 8. 97 % respectively relative to the heterotrophic count. This observation is similar to the results of Ibegbulam -Njoke et al. (2014) who reported that THB had the highest count in similar study involving POME, while POME-utilizing bacteria (PUB) had the second highest population, which was in turn higher than the count for lipolytic bacteria. This decreasing trend in count is justifiable as the total heterotrophic bacteria community of the effluent may likely contain other microbes which may not be able to utilize the effluent and the PUB and LPB were therefore fractions of the heterotrophic community. Results also infer that effluent utilizing bacteria may not necessarily be lipolytic bacteria; as nutrients may be derived from other components of the effluent. Palm oil mill effluent harbors a wide array of microorganisms including POME- utilizing bacteria which are also lipase- producing. Lipolytic bacterial isolates obtained in this study were two strains of *Bacillus circulans*, *Brenneria nigrifluens*, and *Paenibacillus pectinilyticus*. This finding corroborates the report of Soleimaninanadegani and Manshad (2014) who isolated *Bacillus* spp. as lipolytic bacteria from POME. Palm oil mill effluent is an agro-industrial waste generated from the processing of oil palm fruit. Therefore, the isolation of lipolytic *Paenibacillus pectinilyticus* in this study agrees with the finding of Pham *et al*. (2021) who isolated lipolytic *Paenibacillus* spp. from agricultural waste. These organisms belong to both Gram positive and Gram negative bacterial genera. The presence of *Brenneria nigrifluens* (Gram negative) suggests that both Gram positive and Gram negative bacteria can produce lipase and utilize POME as carbon and energy source. Patel and Parikh (2022) also listed *Bacillus* spp., *Pseudomonas*, *Serratia* and *Burlkhoderia* which are both Gram positive and Gram negative as some important bacterial species that produce extracellular lipase.

The pH of the POME sample used in this study was 5.03 suggesting the acidic nature of the effluent which may result from the constituent organic solids or fatty acid developed from the hydrolysis of lipid content or partial degradation of palm fruits before oil extraction and processing of POME. The pH obtained in this study is within the pH of 3.4 - 5.2 reported for POME by Mohammad *et al.* (2021). The acidic pH also suggests that only acid-tolerant bacteria can grow in POME. The isolation of lipase – producing bacteria from the effluent agrees with the report of Gupta *et al*. (2004) that bacteria lipases are stable in a wide range of pH from 4 - 11 and suggesting why the population of PUB and LPB were relatively low. Guan and Liu (2023) ascribed the growth of acid-tolerant bacteria in acid stressed environment to the development of several survival mechanisms by the bcteria. The relatively low population of lipase-producing bacteria in POME sample can therefore be ascribed to the acidic nature of POME. This assertion is in agreement with the report of Gupta et al. (2004) and Ilesanmi *et al.* (2020) which reported alkaline pH of 7-11 for high lipase.

The POME utilization potential of the individual strains and consortium revealed that all the isolates and consortium have varying potential for the utilization of POME as sole source of carbon and energy. However, apart from the bacterial consortium with OD of 0.318 at day 6, *Bacillus circulans* (93. 4 % similarity) among the individual isolates showed highest effluent utilization potential with OD 0.302 at day 6 of growth suggesting its efficiency over the individual isolates in the remediation of the effluent. This variation in palm oil mill utilization is reflected in the differences in optical densities measured with the spectrophotometer at 600 nm. Ikuesan (2017) ascribed the differences in optical density to imply that the pattern of microbial growth and rate of substrate utilization differ from organism to organism. This result agrees with Ganapathy *et al.* (2019) who reported that remediation process of POME involves different microbes that produce lipase enzyme. The results therefore suggest that these lipase – producing bacteria and consortium with ability to utilize POME may be useful as biological agent for the remediation of POME polluted soil and water environments.

The use of *Bacillus* spp. for degradation of palm oil mill effluent (POME) has been reported by Ibegbulam-Njoku *et al.* (2014) and Félicité *et al.* (2021). Palm oil mill effluent is one of the potential sources which may contain lipolytic bacteria. The oily environment had been reported to provide a good source for lipolytic microorganisms to grow. *Bacillus* spp. had also earlier been used in different literature for lipase production in olive mill wastewater and other oil related medium (Veerapagu *et al*., 2013; Ibegbulam-Njoku *et al*., 2014).

Interestingly, *Paenicbacillus* spp. isolated by Pham *et al.* (2021) showed lipolytic activity higher than all *Bacillus* species isolated in their study. However, *Paenibacilllus* was earlier classified in the same group with *Bacillus*; hence, it is not too surprising the species may still show traits similar to *Bacillus* species, but there is still paucity of reports on the lipolytic activity of *Paenibacillus* species especially as relates to palm oil mill effluent. The ability to isolate *Paenibacillus* species from palm oil suggests the lipolytic activity of this organism in POME. There is also paucity of information on the isolation of *Brenerria* species from oil environments or POME. *Brenneria* sp. showed the peaks of effluent utilization potential on day 6 higher than the *Bacillus circulans* strain (LB3). It is therefore suggested that *Brenerria* sp may not be indigenous to palm oil effluent as it has not been reported in any literature to the best of our knowledge as researchers, making this research work novel. However, its lipolytic and effluent utilization ability makes it an organism of interest for further studies as regards bioremediation activities.

Biotreatment of the effluent with individual isolates and consortium resulted in varying reduction of POME quality parameters. The utilization of the palm oil effluent by the isolates and consortium has also been determined by the physicochemical parameters of the raw and treated effluents. The hydrogen ion concentration (pH) was found to increase from acidic (raw) to near neutral and slightly alkaline in the treated effluents, while other quality parameters also reduced. Similar finding as regards pH was recorded by Nwuche *et al*. (2014). Significantly, oil and grease, TSS and TDS, were not detected after treatment as they gave values of 0.00 mg/l (100 % reduction). This supports the findings of previous researchers such as Nwuche *et al.* (2014), who also declared that these parameters were not detected and completely eliminated in treated samples by bacteria strains and mixed culture. Similarly, Chan *et al.* (2010; 2012) had reported 99.00 % removal of TSS using respective different biological treatment of POME.

Other quality parameters such as chemical oxygen demand and biological oxygen demand showed varying percentage reduction suggesting that the efficiency of the isolates to metabolize POME differs. This implies that different microorganisms metabolize pollutants at different rates which may be influenced by genetic or environmental factors or factors due to the nature of pollutant. Importantly, results revealed that except for TOC, the microbial consortium showed greater reduction efficiency for all the parameter. This implies that microbial interaction among the constituents of the consortium can lead to synergy or enhanced degradation rates and maximum substrate utilization. The findings of this research indicated that lipolytic microorganisms isolated in this study are able to utilize the palm oil mill effluent at different rates, hence their potential in bioremediation. This assertion agrees with Ganapathy et al. (2019) who stated that bioremediation specifically involves different microbes that produce lipase enzyme in the oil effluent remediation process.

CONCLUSION AND RECOMMENDATION

Palm oil mill effluent is considered a high-strength pollutant with low pH due to the organic and free fatty acids resulting from partial degradation of palm fruits before oil extraction and processing. Lipase producing bacteria isolated from this study belong to both the Gram positive and Gram negative groups with the Gram positive dominating. This research indicated that POME indigenous lipase-producing bacteria improved POME quality at different rates and therefore suggestive of their potentials in the bioremediation of POME contaminated environment with the consortium being most promising. Lipase enzyme from these bacteria is suggested for commercial production and purification for use in POME remediation. The isolates have been able to utilize palm oil effluent and therefore can be engaged as microbial agents for the bioremediation of palm oil mill effluent. Consortium rather than single strain organisms should be engaged in the bioremediation of effluents.

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