Use of Palm Oil Mill Effluent as Fermentative Medium by Lipase Producing Bacteria

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Abstract— This work was carried out to evaluate palm oil mill effluent (POME) as a fermentative medium for lipase production at laboratory scale using two bacterial isolates (*Bacillus cereus* and *Pseudomonas aeruginosa*). The physicochemical analysis of raw POME showed high organic load of COD (78750 mg/l), BOD (15050 mg/l), oil and grease (7645 mg/l), TS (4125 mg/l) and TSS (1650 mg/l).The extracellular lipase producing bacteria *Bacillus* cereus, *Proteus vulgarius*, *Pseudomonas aeruginosa* and Staphylococcus *aureus* were isolated from palm oil mill industrial effluents. Optimization of physical and chemical factors was done to improve lipase production using the respective bacterial isolates. Percentage biomass improvement, Oil and grease (O&G) reduction and lipase activities were measured with varying pH (4-10), incubation temperature (20-55°C), incubation time (144hrs.), carbon source concentration, nitrogen source concentration, and various metal ions. Enhanced lipase activity for both isolates was observed at 35°C, pH 6&7 for *Bacillus cereus* and *Pseudomonas aeruginosa* respectively, after 144hrs of incubation. Corn meal best supported the activities of *Bacillus cereus* and *Pseudomonas aeruginosa* at respective concentrations of 3.5wt/v and 2.5wt/v. Soy meal at concentration 2.5wt /v supported the lipase activities of both isolates while Ca^{2+} showed good lipase activity among the metal ions with both isolates. The present study showed increased lipase activities of both bacterial isolates and demonstrated POME as very good source of fermentation medium for production of extracellular lipases.

Keywords — Biodegradation, BOD, COD, Lipase, Oil and grease, Palm oil mill effluent

INTRODUCTION

Palm Oil Mill Effluent (POME) is a highly voluminous liquid waste with high content of carbohydrates, protein, nitrogenous compounds, lipids and mineral) which in turn releases unpleasant smell [1].

The high organic content in POME makes it possible to reuse the effluent for biotechnological purposes [55]. Oil wastes are considered serious pollutants when discharged into aquatic environments. Wastewaters containing fat and oils were previously treated physically and this is considered incomplete when the fat is in a dispersed form. In recent time, biological treatment has been found to be a more effective method by removing fat, oil and grease by degrading them into miscible molecules [15]. Therefore, modification of microorganisms and use of lipase enzymes produced by organisms for treatment and bioremediation purposes may solve the problem of purifying contaminated effluent and large bodies of water.

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolyses of long chain triglycerides to intermediate and short chain glycerides, free fatty acids and glycerol [44, 5].

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2 Material &method

2.1 Experimental Sample:

Raw POME obtained from Star-line Palm Oil Mill Industry, Umukalika, Obingwa, Abia State Nigeria was collected from a discharge of acidification pond in sterile plastics bags, stored at 4^0C and transported to the laboratory for analyses.

Lipases are also involved in a lot of conversion reactions such as esterification, transesterification, alcoholysis, acidolysis and aminolysis in non aqueous media [47]. The usefulness of lipases makes the enzyme very important in food, pharmaceutical, textile, paper and cosmetic industries [21].

The application of lipase producing microorganisms into effluent treatment system to degrade fat and oil have attracted considerable attention owing to their biotechnological potential, ranging from the use in laundry detergents to stereospecific biocatalysis. Thus, there is growing interest in discovering new sources of these enzymes with appropriate characteristics to suit particular applications[4]. The majority of commercially produced lipases originate from fungi (genera *Rhizomucor*, *Rhizopus* and *Candida*) and bacteria (genera *Pseudomonas* and *Chromobacterium*)[48 and 30]. Studies on biotreatments of palm oil mill effluent by selected lipase producing bacteria were carried out. Lipase production from bacterial isolates has been very important in the aspect of finding novel lipases with specific properties along side lower production and processing cost.

The present study is aimed at assessing palm oil mill effluent as a fermentative medium for lipase production at laboratory scale.

2.2Physicochemical characteristics of Palm Oil Mill Effluent (POME)

The raw POME collected was analyzed accordingly for total suspended solids (TSS), total dissolved solids (TDS), turbidity, pH, chemical oxygen demand (COD), Biochemical oxygen demand and oil & grease using standard methods [3]. The TSS, TDS were determined using portable hand held meter (HANNA-DIST 1 HI 991002) while turbidity and pH of the samples were determined using Unicam8625 spectrophotometer (at 340nm wave length) and laboratory pH meter (HANNA HI 9820) respectively. The temperature of the effluent sample was determined using a laboratory thermometer. The Chemical Oxygen Demand (COD) was determined by titrimetic method as described in standard method for the Examination of water and wastewater APHA1998 using Ferrous ammonium sulphate ($(NH_4)_2SO_4$. $FeSO_4.6H_2O$) and biological oxygen demand (BOD_5) was determined using HACH model 2173 BOD measurement apparatus).Oil and grease was determined using gravimetric method after soxhlet extraction. Three repeatable experiments were tested and the average values with their standard deviations were recorded.

2.3 Screening for lipase producing organisms.

The Isolation of lipolytic microbes was described by [11 and 27] using 1.0g of soil sample collected from different location of the palm oil mill site and serially diluted with sterile distilled water, spread on the sterilized nutrient agar plates followed by incubation for 24-48h at 37 °C . Observed microbial colonies were purified and subjected to qualitative screening test for lipase producing micro-organisms on tributyrin agar. Tributyrin agar was sterilized at 121°C for 15mins and cooled to 45°C. Holes were bore on the solidified agar plates and inoculated with 0.1ml of 10^{-3} serially diluted isolates in physiological saline solution into the hole, allowed to diffuse and incubated at 37°C for 48hrs. Zones of clearance signified positive lipolytic organisms. Microbes with large zones of clearance where further identified using physiological, morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology[17].

2.4 Microorganism and enzyme production.

The method used was described by [20, 18] with slight modification using POME as fermentation medium. The selected bacterial isolates were respectively grown in 100ml palm oil mill effluent sample contained into 250ml Erlenmeyer flasks and inoculated with 0.1ml (10^6cells/ml) of selected culture respectively. The flasks were incubated at 28° C for 144h on a rotary shaker with agitation speed of 180rpm. The lipase enzyme produced at optimum conditions were precipitated and crude enzyme used for purification process as described by [37].

2.5 Determination of Biomass.

The biomass was determined as described by [54] with slight modification using cell dry weight measurements of 100ml culture sample. The samples were filtered through dried and pre-weighed filter paper (Whatman No.1), followed by washing thrice with distilled water and then drying at 60°C to constant weight. The cell free samples were further centrifuged at 13,000 x g for 15min. The supernatants were kept at 4°C for further analysis.

2.6 Lipase Assay (Titrimetric method).

The lipase activity in crude extract of respective selected bacterial isolates was measured using titrimetric method as described in previous work but with slight modification of cation [10,2]. The assay for extracellular lipase was carried out using Palm oil as a substrate. Palm oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 100 mM potassium phosphate buffer at pH 7.0. Partially purified lipase (10 µl) was added to the emulsion and incubated for 30 min. at 37°C. The reaction was stopped and fatty acids were extracted by addition of 1.0 ml of 1:1 acetone/ ethanol solution. The amount of fatty acid liberated was estimated by titrating with 0.05M NaOH using phenophathelin. Blank assays were conducted adding the enzyme just before titration.

One unit (u) of lipase activity was defined as the amount of enzyme required to hydrolyze 1µmol of fatty acids from triglycerides indicator under assay condition [42]

Lipase activity =

$$\frac{\text{Vol. of NaoH consumed (ml)} \times \text{Molarity of NaoH}}{\text{Vol. of Lipase (ml)} \times \text{Reaction Time (min)}}$$

One unit of lipase activity was defined as the amount of enzyme that liberated 1µmol fatty acid min-1 at 30 °C at pH 10.5 under the assay conditions [42].

2.7 Effect of initial pH on lipase activity of bacterial isolates in POME.

The method used was described by [54 and 14] with little modification in palm oil mill effluent. The effect of pH on lipolytic activity was determined using the following buffers (all at 50 mM): succinate-NaOH (pH 4.0 and 5.0), sodium phosphate (pH 6.0 and 7.0), Tris–HCl (pH 8.0 and9.0) and glycerin-NaOH (pH 10.0) to improve initial pH of production medium (palm oil mill effluent sample) contained into 250ml Erlenmeyer flasks and inoculated with 0.1ml (10^6cells/ml) of selected culture respectively. The flasks were incubated at 28° C for 144h on a rotary shaker with agitation speed of 180rpm then crude enzymes washed and precipitated for determination of lipase activity.

2.8 Effect of incubation temperature on lipase activity of bacterial isolates in POME degradation.

The method used was described by [28] with slight modification in temperature of incubation. The crude enzyme used for assay was the culture broth (POME) after separation of cells and particles. The enzyme was

normally stored at 4°C until used. The enzyme assay was performed as discussed above except that incubation was done at temperatures 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C and 55°C respectively at pH 8.0 for 30 min.

2.9 Effect of carbon concentration on lipase activity of bacterial isolates in POME degradation.

The method used was as described by[45] with little modification in palm oil mill effluent. Respective corn meal concentrations (1.5, 2.5, 3.5and 4.5% w/v) were used to modify100ml of palm oil mill effluent sample in 250ml Erlenmeyer flasks. It was further inoculated with selected bacterial isolate (10^6cells/ml) and incubated at 28° C for144h on a rotary shaker with agitation speed of 180rpm. Samples were drawn for determination of lipase activity.

2.10 Effect of nitrogen concentration on lipase activity of bacterial isolates POME degradation.

The method used was as described by [45] with little modification in palm oil mill effluent. Palm oil mill effluent sample (100ml) was modified with respective concentrations of soy bean (1.5, 2.5, 3.5and 4.5% w/v) and inoculated with 0.1ml (10^6cells/ml) of selected inoculums. The flasks were incubated at 28° C for 144hrs on a rotary shaker with agitation speed of 180rpm. Samples were drawn for determination of lipase activity.

2.11 Effect of metal ions on lipase activity of bacterial isolates in PO ME degradation.

The method used was described by [20] with slight modification in fermentation medium and metal sources. Different metal ions (Na^{2+}, K^{2+}, Ca^{2+}, Mn^{2+}, , Mg^{2+}, Cu^{2+}, Fe^{3+}, Ag^{+}) – 0.1%(w/v) salts were incorporated into 100ml of palm oil mill effluent samples contained in 250ml Erlenmeyer flasks at 0.1% (w/v) concentration, pH 6 and later inoculated with 0.1ml (10^6cells/ml) selected inoculum. The flasks were incubated at 28° C for 144h on a rotary shaker with agitation speed of 180rpm. Samples were drawn for determination of lipase activity.

3.0 RESULTS

3.1 Physicochemical characteristics of palm oil mill effluent.

The physicochemical analysis of POME from Starline palm oil mill Industries Umukalika, Obingwa, Abia State, Nigeria is presented in Table 1. The mean values are 3.86 (pH), 78750 mg/l (COD), 15050 mg/l (BOD), 4125 mg/l(TS) 81mg/l(PO_4^{3-}), 1650 mg/l(TSS) 7645 mg/l (oil and grease), 71mg/l (Na^{2+}), 78 centipoises (Viscosity), 34 ° C (Temp) , 915 mg/l(TKN), 144.5 mg/l(NH_3N) and 198.5mg/l(FFA).

Table 1. Physicochemical analysis of raw palm oil mill effluent samples from Starline Palm Oil mill Industries.

Properties	Range	Mean
COD (mg/l)	69000 - 90000	78750±18
BOD (mg/l)	12000 - 18200	15050±25
Oil/ grease (mg/l)	7300 - 7860	7645±24
TS (mg/l)	4000 - 4400	4125±18
TDS (mg/l)	1975 - 2175	2096±85
TSS (mg/l)	1500 - 1800	1650±12
FFA (mg/l)	147.6 - 277.2	198.5±56
TKN (mg/l)	780 - 1000	915 ±99
NH_3N (mg/l)	127 -174	144.5±20
pH	3.74 - 3.98	3.86±0.11
Temp (° C)	32 - 35	34±1.26
Viscosity	76 - 80	78±1.83
Phosphate	81 - 83	81±1.26
Alkalinity (mg/l)	50 - 58	50±6.53
Sodium	69 - 73	71±2.31

Table 2 Screening for bacterial isolates from palm oil mill effluents for lipase production.

Organisms	Zones of clearance
Bacillus cereus	34
Pseudomonas aeruginosa	36
Mucor racenosus	12
Proteus vulgarius	10
Staphylococcus aureus	18

Screening for lipase producing bacterial isolates from palm oil mill effluents was demonstrated in table 2. The test for zones of clearance in five bacterial isolates revealed that *Pseudomonas aeruginosa* and *Bacillus cereus* showed higher zones of clearance of 36 and 34mm respectively while the least (10mm)was shown by *Proteus vulgaricus*

The effect of initial pH of Culture Broth during biodegradation of POME was demonstrated in Fig 1 & 2 using respective bacterial isolates (*Bacillus cereus* and *Pseudomonas aeruginosa*). In Fig 1, *Bacillus cereus* showed greater capacity in oil and grease reduction from POME at pH range of 6 to 8. Best performance of *Bacillus cereus* was observed at pH 6 with lipase activity of 41U/ml, oil and grease reduction of 50.5%. The highest growth rate was at pH 7 with 66.6% Biomass. However, Initial pH of 10, showed the least support for growth/biomass (23%) oil and grease reduction (14.1%) and lipase activity(6U/ml).

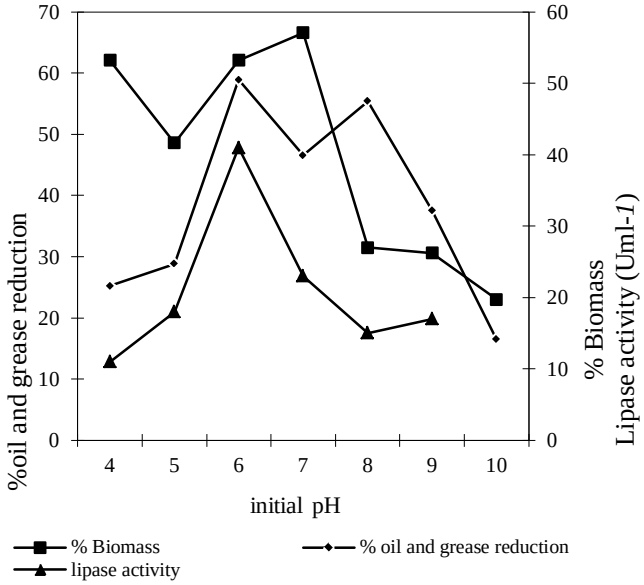

Figure 1. Effect of initial pH on %Biomass, lipase activity, %oil and grease reduction in Bacillus cereus degraded POME.

The effect of initial pH on % oil and grease reduction, lipase activity and % biomass in *Pseudomonas aeruginosa* degraded POME was shown in Fig 2. The effect of initial pH culture broth (POME) on the lipase activity was determined using four different buffers covering the range of pH 4.0 to 10.0 .The results showed that the *Pseudomonas aeruginosa* was able to grow in the pH range of 6 to 9. An initial pH of 7 best supported biodegradation of POME with biomass of 61%, oil and grease reduction of 47% and lipase activity of 38 U/ml. The least support for biodegradation of POME using *Pseudomonas aeruginosa* was shown at pH 10 with lipase activity of 38 U/ml, oil and grease reduction of 19.6 and biomass 32%.

Effect of incubation temperature on lipase activity of microbial isolates.

Lipase activity at different temperatures ranging from 20°C to 55°C is shown in (Fig 3 & 4). Temperature of 35°C best supported the degradation of POME using both selected bacterial isolates. *Bacillus cereus* showed maximum lipase activity (46 U/ml), biomass (62%), oil and grease reduction (64%) (Fig 3). Similarly, *Pseudomonas aeruginosa* showed lipase activity of 48U/ml, biomass (57%), oil and grease reduction 74% (Fig 4). However, a temp of 20 °C showed the least support for *Pseudomonas aeruginosa* degraded POME with lipase activity of 23U/ml, biomass (28%), oil and grease reduction (39%).

The effect of metal ions on activity of lipase.

The effect of different metal ions (Na^{2+}, K^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Ag^{+}) on lipase activity of bacterial isolates is shown in Fig 9 & 10 . The results indicated that K^{2+}, Ca^{+2}, Mg^{+2} ions activated the lipase activity by different degree (Fig 9). *Bacillus cereus* best utilize Ca^{+2} ions in POME degradation with oil and grease reduction of 58%, biomass 36% and lipase activity of 37U/ml while the least support was observed in Ag^{+} ions with 28% biomass, 18% oil and grease reduction and 17 U/ml lipase activity.

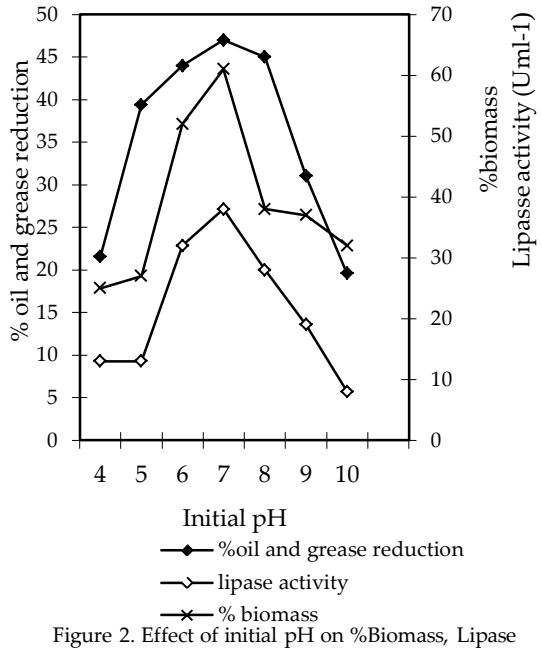

Figure 2. Effect of initial pH on %Biomass, Lipase activity, oil and grease reduction in Pseudomonas *aeruginosa* degraded POME

Effect of carbon and nitrogen concentrations on lipase activity of microbial isolates.

The effect of carbon concentration was represented in Fig 5&6 using corn meal as source of carbon. In Fig 5, *Bacillus cereus* best utilized corn meal at concentration of 3.5wt/v with %biomass (58), lipase activity 52U/ml, and oil and grease reduction of 63%. The lowest support was observed at 1.5wt/v of corn meal with lipase activity of 14U/ml, biomass (27%), oil and grease reduction of 11%. Also corn meal best supported the growth of *Pseudomonas aeruginosa* at concentration of 2.5wt/v with improved biomass (49%), lipase activity of 42U/ml, oil and grease 48%. However, the lowest concentration (1.5wt/v) showed the least support for activities of *Pseudomonas aeruginosa* in POME.

Similarly, Fig 7 & 8 demonstrated the use of soy bean as the organic nitrogen source at various concentrations. The result indicated that at 2.5wt/v of soybean *Bacillus cereus* activity was best supported with 58% oil and grease removal from POME, 66% biomass and 42 U/ml lipase activities. Similarly, maximum lipase activity of 55U/ml was significantly observed by soy bean at concentration of 2.5wt/v in *Pseudomonas aeruginosa* degraded POME with improved biomass (57%), and an oil and grease reduction of 69%. However, Soy bean concentration of 1.5wt/v showed the least support in the activities of both *Bacillus cereus* and *Pseudomonas aeruginosa*.

DISCUSSION

Physicochemical characteristics of palm oil mill effluent

The result of this study is agreement with findings of [1, 46] that reported a pH of 4.7 and the mean temperature obtained is also in tandem with the findings of [39 and 40] that reported atmospheric temperature during oil palm processing in the range of 27.23 –35.60ºC. COD showed a high value of 78750mg/l and this compared favourably with previous reports of [12], other authors reported average COD of 50,00mg/l [7, 3]. An average BOD value of 15050 mg/l reported contradicts reports of [3,53] but agrees with report of [41] of BOD(11,000mg/l). BOD values of raw POME sample used in this study was higher than the IFC (2007) guideline value of 50mg/l for vegetable oil processing effluents. Therefore, the discharge of POME into the ecosystem could result to pollution and hence necessitated this study on how to control the pollution effect in the environment.

Screening for bacterial isolates from palm oil mill effluents for lipase production

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[37]. In this study, lipolytic organisms were cultured in palm oil mill effluent at different treatment conditions. *Pseudomonas sp* is known to be ubiquitous and capable of utilizing a wide range of simple and complex organic compounds. It is therefore considered a very important scientific and technological tool in industries [35]. Similarly, *Bacillus* sp had been noted for its importance in the enzyme industry [8]. The following organisms *Bacillus sp*, *Pseudomonas sp*, *Proteus* sp, *Staphylococcus, Geotricum candidium, Candida* sp *and Mucor* sp had earlier been used in different literature for lipase production in olive mill wastewater and other oil related medium but not palm oil mill effluents[49, 16, 41]. The use of Bacillus sp and Pseudomonas as lipolytic organisms have been reported in some literature [43], however, to the best of our knowledge prior to this study , Bacillus cereus and *Pseudomonas aeroginosa* have not been used for degradation of palm oil mill effluent.

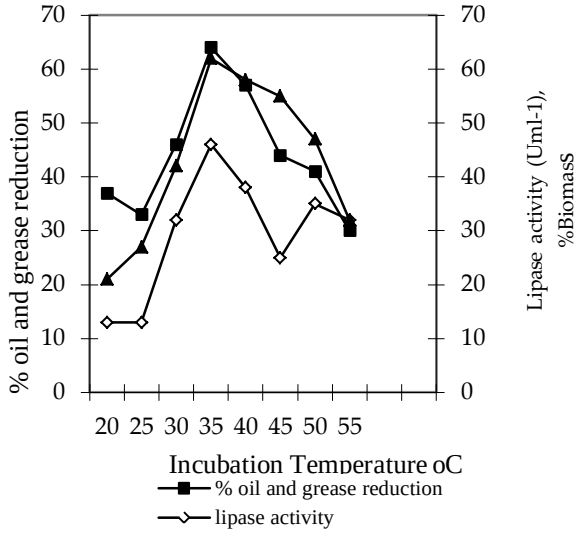

Figure 3. Effect of incubation temperature on %Biomass, lipase activity, % oil and grease reduction in *Bacillus cereus* degraded POME.

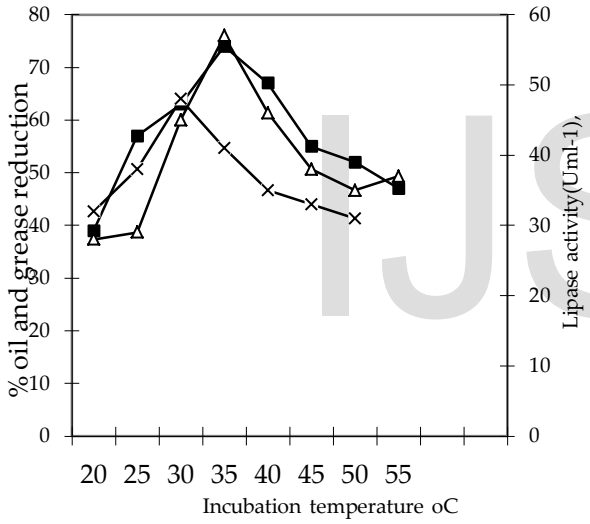

Figure 4.Effect of incubation temperature on % Biomass , lipase activity, % oil and grease reduction in *Pseudomonas aeruginosa* degraded POME.

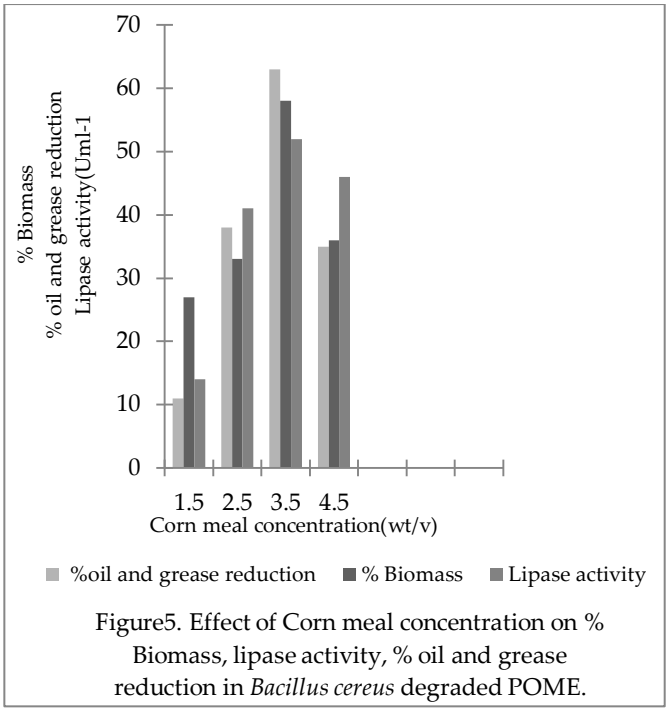

Figure5. Effect of Corn meal concentration on % Biomass, lipase activity, % oil and grease reduction in *Bacillus cereus* degraded POME.

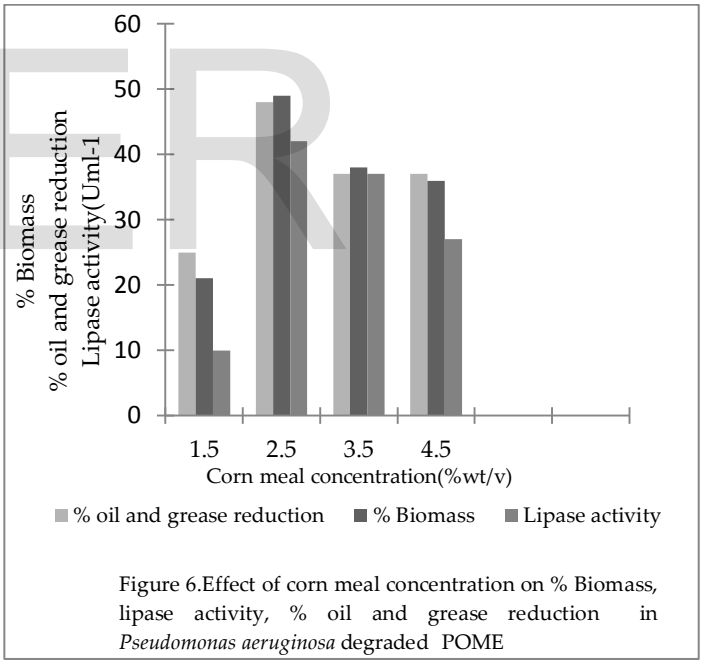

Figure 6.Effect of corn meal concentration on % Biomass, lipase activity, % oil and grease reduction in *Pseudomonas aeruginosa* degraded POME

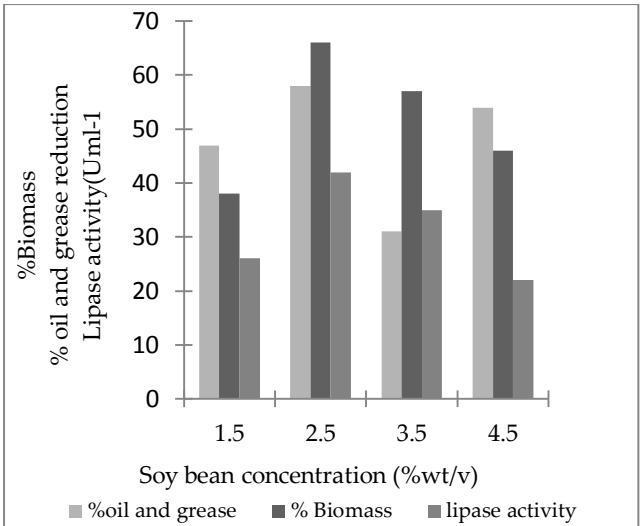

Figure7. Effect of soybean concentration on %biomass, lipase activity, % oil and grease reduction in *Bacillus cereus* degraded POME.

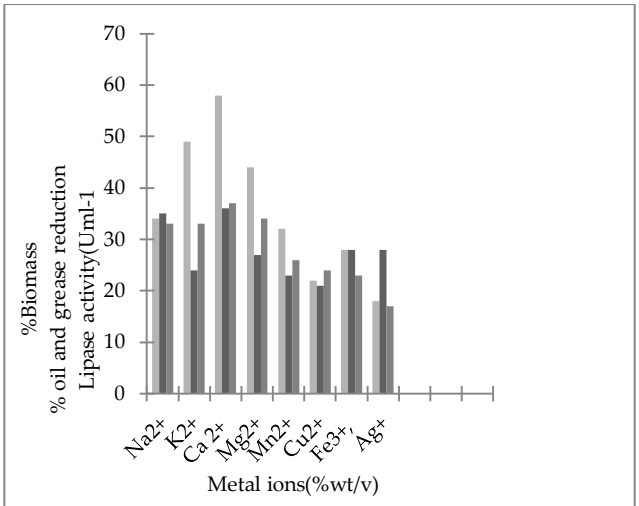

Figure 9. Effect of metal ion on % Biomass, lipase activity, oil and grease reduction in Bacillus cereus degraded POME .

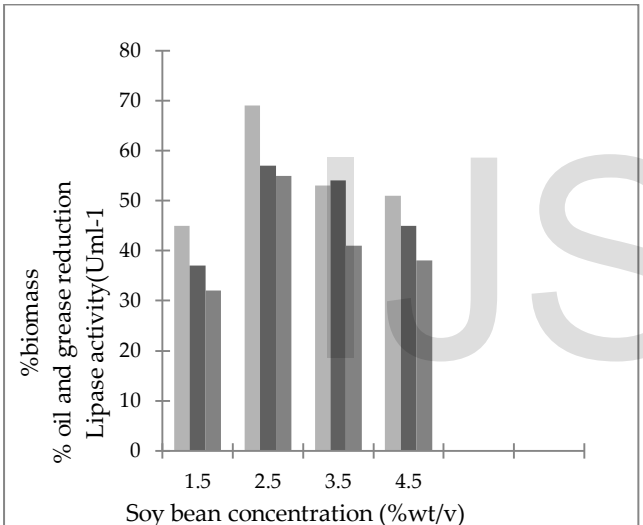

Figure8. Effect of soy bean concentration on % biomass, lipase activity, oil and grease reduction in *Pseudomonas aeruginosa* degraded POME

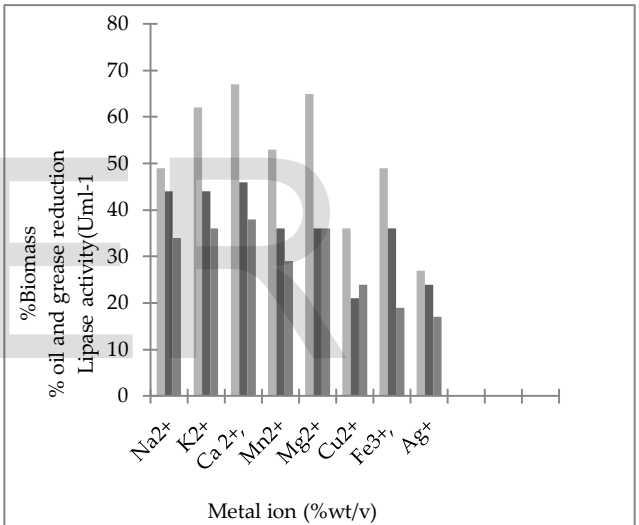

Figure10. Effect of metal ion on% Biomass, lipase activity, % oil and grease reduction in Pseudomonas aeruginosa degraded POME

Effect of initial pH on lipase activity of bacterial isolates in POME degradation.

The pH of the cultural medium has an important role for the optimal physiological performances of the bacterial cell and transport of different nutrients across the cell membrane aiming at maximizing the enzyme yields [37]. Initial pH of the culture broth is one of the most critical environmental parameters affecting both growth and lipase production[6]. The effect of initial pH on culture broth (POME) was demonstrated in Fig 1& 2 showing *Bacillus cereus* to have greater capacity degraded oil and grease in POME at pH range of 6 to 8 with greatest performance at pH 6 .The result showed lipase activity of 41U/ml, oil and grease reduction of 50.5% and highest growth rate (66.6%) at pH 7. The result is in agreement with report of [36] which demonstrated maximum lipase activity at pH of 6. However, initial pH of 10, showed the least support for growth/biomass (23%) oil and grease reduction (14.1%) and lipase activity (6U/ml). Previous reports revealed lipase activity from some Bacillus species [(38, 32, 31)] at optimal pH of 8-9 while others [51] reported *Bacillus specie* lipase at maximum activity of pH 5.6.

The growth of *Pseudomonas aeruginosa* was highly supported at pH range 6-9 (Fig 2) and reached the maximum lipase activity of 38 U/ml, Biomass 61%, oil and grease 47% at pH 7. The increasing activity from pH 6-9 can be characterized as

an alkalophilic enzyme but lipase activity at pH 6 can be applicable to acidic conditions [26]. The result is in agreement with that of Yuzo *et al.,* 2003 who reported that maximum lipase activity from *Pseudomonas fluorescens* HU 380 was detected at pH 7. Similarly, previous reports had shown that most bacterial lipases are stable in a wide range of pH from 4 to 11 and are capable of yielding maximum lipases activity at pH values above 7 [19]. Many work had shown bacterial isolates such as Pseudomonas fluorescens HU 380 [29], *P. mendocina* PK-12CS [24] and *P. pseudomalei* 12 sm [25] as alkaline lipase activities.

Effect of incubation temperature on lipase activity of bacterial in POME degradation.

Lipase activity at different temperatures ranging from 20°C to 55°C is shown in (Fig 3&4). Temperature of 35°C best supported the degradation of POME using the respective bacterial isolates (*Bacillus cereus* and *Pseudomonas aeruginosa*). However, temp of 20 °C showed the least support for *Pseudomonas aeruginosa* degraded POME with lipase activity of 23U/ml, biomass (28%), and oil & grease reduction (39%).

The result concurs with the report of [43] who revealed lipase activity (8.321U/ml) by *Pseudomonas aeruginosa* at 35°C. The optimum temperature of 30°C for lipase production was shown to produce lipase activity of 0.62 U/ ml in Bacillus [37]. The other bacterial strain had shown maximum lipase activity at temperature 30°C [50,6].

Effect of metal ions on lipase activity of bacterial isolates in POME degradation.

The effect of various metal ions used showed that K^{2+}, Ca^{+2}, Mg^{+2} ions activated the lipase activity by different degree (Fig 9.) but *Bacillus cereus* best utilize Ca^{+2} ions in POME degradation with improved oil and grease reduction of 58% and lipase activity of 37U/ml. Similarly, *Pseudomonas aeruginosa* best utilized Ca^{+2} ions in degradation of palm oil mill effluent with oil and grease removal of 67%, 46 % Biomass improvement and lipase activity of 38U/ml. Reports show that these ions play an important role as lipase cofactor [22, 6] . Effect of the heavy metal ions Ag^{+} and Cu^{2+} on lipase activity in this work was similar to their effect on lipases from *Pseudomonas aeruginosa* LP602[13], P. *fluorescens* 2D[33] and HU380 (Kojima 2003). Reports have shown that the effect of metal ions could be attributed to a change in the solubility and the behavior of the ionized fatty acids at interfaces [34].

CONCLUSION

The ability of palm oil mill effluent to serve as a good source of fermentation medium for improvement of lipase activities in both bacterial isolates under various experimental conditions makes it very resourceful rather than constitute pollution in the environment. Hence the government can assist scientists in this area of research to produce this enzyme in mass.

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