



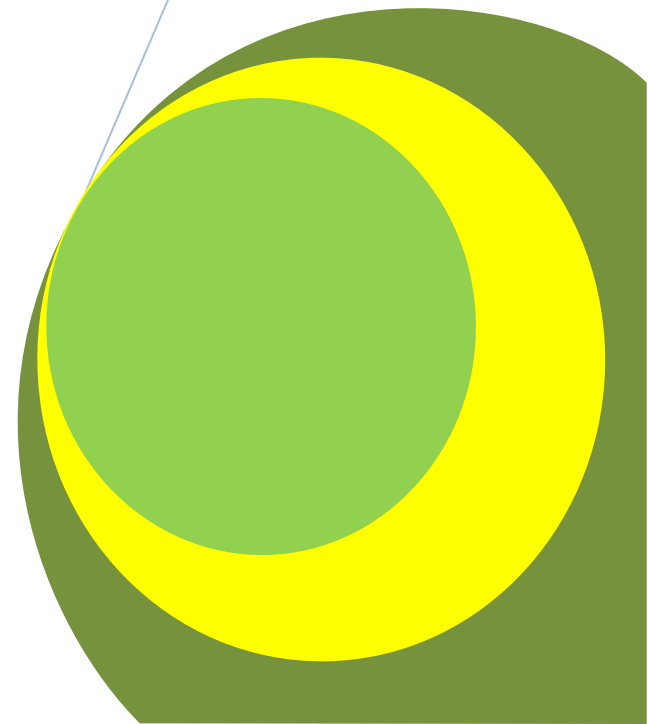
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Physicochemical and Microbial Screening of Palm Oil Mill Effluents for Amylase Production

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Research Article

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ABSTRACT

With the advent of biotechnology, several industries are dependent on enzyme for production processes. This study evaluated the physicochemical and microbiological quality of POME for amylase production. Triplicate POME samples were collected aseptically from semi-mechanized oil palm processing mills in Bayelsa state, Nigeria. The physicochemical properties of the POME samples were determined using standard analytical procedures. The results of the physicochemical quality assessment result were 6.56 (pH), 4.69 mg/l (DO), 1806.33 mg/l (COD), 382.93 mg/l (BOD), 8.18 mg/l (PO_4^{3-}), 19.64 mg/l (K), 12.87 mg/l (N), 73.07 mg/l (oil and grease), 0.03 mg/l (Cd), 2.44 mg/l (Cu), 5.62 mg/l (Fe) and 2.01 mg/l (Cr). The microbial populations of the samples were 1.3×10^5 cfu/ml and 6.9×10^3 cfu/ml for total heterotrophic bacteria and total fungi respectively. The bacteria isolates are *Micrococcus* species, *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, while the fungi isolates are *Aspergillus niger*, *Aspergillus fumigatus*, *Candida* species, *Fusarium* species, *Mucor* species and *Penicillium* species. Of bacterial isolates, *Micrococcus* species and *Staphylococcus aureus* has the highest and least occurrence frequency of 34% and 13% respectively, while in fungi isolates *Penicillium* species and *Fusarium* species has the highest and least isolates with 23% and 7% respectively. Of the microbial isolates of POME, *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, *Penicillium* species, *Fusarium* species, *Mucor* species, *Candida* species and *Aspergillus niger* were predominant for the production of amylase. This amylase can be utilized in several industrial/biotechnological sectors including biofuels.

Keywords: Amylase, biotechnology, lipase, microbial amylase, palm oil mill effluents (POME), physicochemical.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq) is a versatile monoecious oil bearing plant that thrives in tropical and subtropical regions of the world. Oil palm requires climatic condition of 1800 – 5000 mm/year (rainfall) (Embrandiri et al., 2012), 17 - 28°C (temperature) and >75% (relative humidity) (Poku, 2002). Oil palm is propagated by seed. It requires nursery for a period of 9 – 12 months before being planted in the plantation. The economic plant starts bearing fruit at 3 to 5 years and reaching optimal yield at 10 years from planting. Oil palm has an economic life value of 20 – 30 years and life span of about 200 years. Global oil palm cultivation has been dominated majorly by Indonesia and Malaysia and to a lesser extent by Colombia, Thailand and Nigeria. These countries combined to produce over 93% of global oil palm output. In Nigeria, about 80% of the oil palm industry is dominated by smallholders who typically use rudimentary equipment for processing (Ohimain et al., 2012a, b) and to a lesser extent by semi-mechanized processors. The mechanized processing mills are located mainly in southern Nigeria where the oil palm is found in both wild and plantations. The fresh fruit bunch (FFB) is harvested from the plant and transported to the mill. During oil palm processing activities bruises occur leading to microbial infestation of the palm fruit, which enhanced the proliferation of lipolytic microorganisms that flourishes in crude palm oil. The action of these microorganisms leads to high free fatty acid content. Ohimain et al. (2012b), Izah and Ohimain (2013a) reported that high free acid found in palm oil lead to deterioration of quality especially their industrial application.

During oil extraction from FFB, three wastes streams are generated; solid (empty fruit bunch, palm press fiber and chaff) (Ohimain et al., 2013a; Rupani et al., 2010), liquid (palm oil mill effluents) (Ohimain et al., 2012c; Ohimain and Izah, 2013a; Ismaili et al., 2010; Ugoji, 1997) and gaseous wastes (Ohimain et al., 2013b). Of particular concern is POME which has caused several environmental problems. Sridhar and AdeOluwa (2009), Awotoye et al. (2011) reported that the discharge of untreated POME into the ecosystem leads to the loss of biodiversity, soil deterioration and pollution of waterways. POME released to aquatic ecosystem turns water brown and smelly (Awotoye et al., 2011). The smelly nature of POME causes odor pollution (Er et al., 2011). To a large extent, pollution

associated with POME discharge is due to high biological oxygen demand (BOD), chemical oxygen demand (COD), oil and grease it contain. POME contains complex polymers (carbohydrates, protein, lipids, minerals and nitrogenous compounds (Habib et al., 1997). POME is naturally thick, brownish containing colloidal slurry of water (95 – 96%), oil (0.6 – 0.7%), total solid (4 – 5%) and suspended solid (2 – 5%) (Onyia et al., 2001; Ahmad et al., 2005). The extraction of palm oil from FFB requires voluminous quantity of water and a large portion of the water end up as POME. Ohimain and Izah (2013a) reported that 72 – 80 liters of water are required to process one tonne of FFB by smallholders in Nigeria. Of these, 72 – 75% ends up as POME. Similarly, Ahmad et al. (2003), Okwute and Isu (2007), Wu et al. (2009) estimated that 5.0 – 7.5 tonnes of POME is generated from the voluminous quantity of water used in the processing of one tonne of FFB.

POME contain a diverse group of microorganisms such as acid forms and hydrocarbon degraders (Ohimain et al., 2012c). Oil palm being a major source of lipase producing microorganisms, several lipolytic bacteria, fungi and mold are capable of flourishing on it (Izah and Ohimain, 2013b), due to its nutrient content. Ohimain et al. (2012d), Wood et al. (1979), Borja et al. (1996) have variously reported micro and macro nutrient as part of POME mineral composition. Microorganisms are able to mineralize these nutrients to produce enzymes. POME microorganisms have found application in bioconversion of POME into useful and demanding products. Wu et al. (2009) stated that POME can be useful in the production of antibiotics, solvent, bio-fertilizer, bio-hydrogen, bio-insecticides, organic acids, polyhydroxyalkanoates and enzymes.

Microorganisms have become increasingly essential as producers of industrial enzymes (Alariya et al., 2013). Enzymes are vital proteins for the metabolic system of living organisms (Alves et al., 2002). Extracellular enzymes such as cellulase, amylase, xylanase, lignin peroxidase, pectinase, β -glucosidase etc can be produced from POME. Amylases are derived from plants, animals and microorganisms (de Souza and Magalhães, 2010; Pandey et al., 2000). Like other extracellular enzymes, amylases are among the most vital enzymes that are useful in present day biotechnology advancement (Alariya et al., 2013; Pandey et al., 2000). Amylases have found applications as additives in detergents, saccharification of starch, food fermentation, textile, paper, fine-chemical, distilling, medical, pharmaceutical (Pathak, and Narula, 2013; Onofre et al., 2012; de Souza and Magalhães, 2010; Pandey et al., 2000), breweries, bioethanol, biodiesel industries. Several substrates have been employed for amylase production but POME has never been reported. Also the physicochemical and microbial quality of POME from semi-mechanized oil palm mill has not been reported. Hence, this study is focused on [a] assessment of microbial and physicochemical quality of POME from semi-mechanized palm oil processing mill in Bayelsa state, Nigeria, [b] screening of the microbial isolates for amylase production.

MATERIALS AND METHODS

2.1. Field Sampling

A semi mechanized oil palm processing mill was visited in Elebele, Bayelsa State, Nigeria on 03– 07 June 2013. Triplicate samples of POME produced from the mill were collected aseptically with sterile microbiological bottles and sampling container for microbial and physicochemical analysis respectively for laboratory analysis. The process of POME extraction from FFB in the oil palm processing mill is presented in Fig. 1.

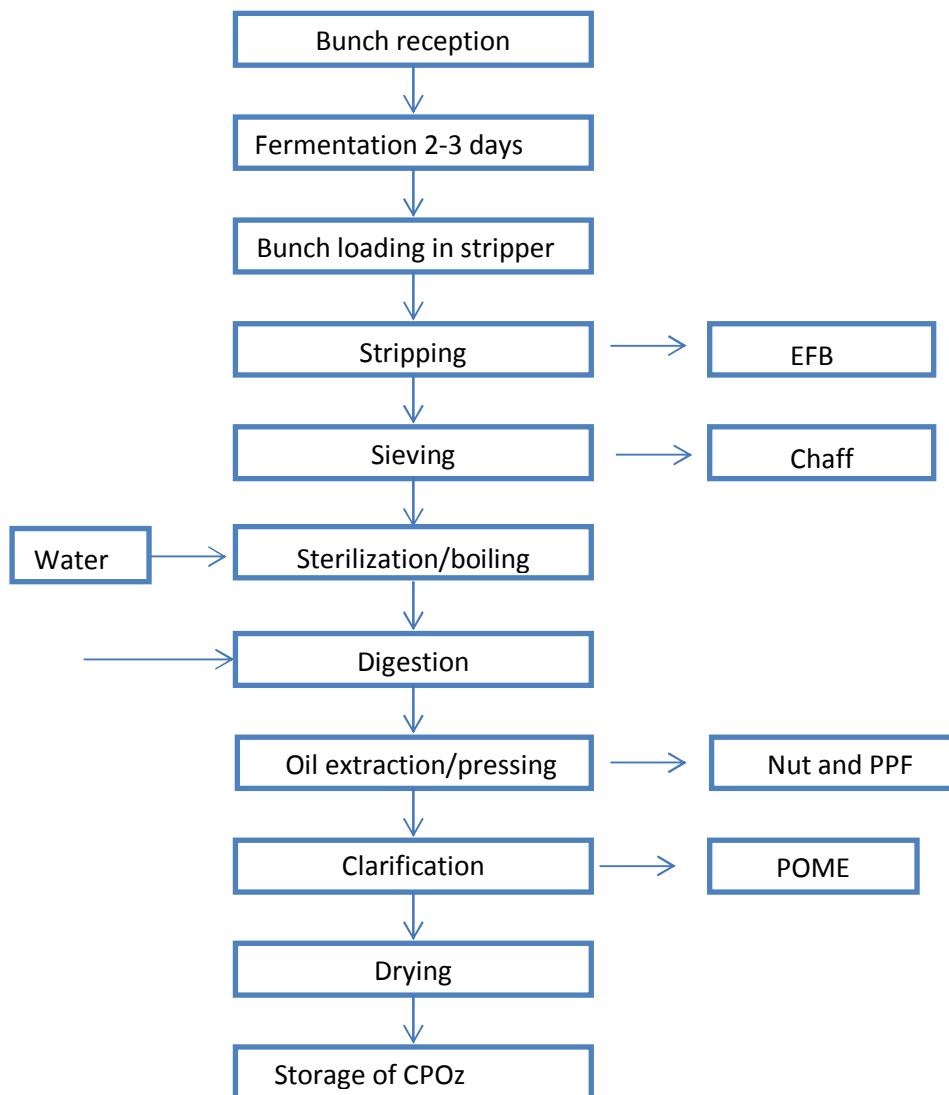


Fig 1: The processes of palm oil processing by semi-mechanized mill in Bayelsa state, Nigeria (POME = palm oil milling effluents; PPF = palm press fibre; CPO = crude palm oil)

2.2 Microbial analysis

2.2.1 Enumeration of total heterotrophic bacteria and fungi

The populations of microorganisms in the samples were enumerated using serial dilution pour plate method of Pepper and Gerba (2004), Benson (2002). About 0.1ml of POME sample was serially diluted in sterile distilled/deionized water and aliquots of the dilutions were aseptically plated into growth media (Nutrient Agar and Sabouraud Dextrose Agar for bacteria and fungi respectively). The agar plates were incubated at 37°C for 24-48 hours to enumerate the aerobe and facultative bacteria and the fungi culture plates were incubated inverted at 30°C for 3-5 days. After incubation, the colonies that grew on the medium were counted and expressed as colony forming units (cfu)/ml of the samples (Ohimain et al., 2012c). Microbial colonies were isolated into pure cultures and preserved in slants for further analysis.

2.2.2 Identification of microbial isolates

The bacteria isolates were identified by biochemical test (gram reaction, motility, indole, catalase, coagulase, oxidase, urease and citrate). The resultant characteristics were compared with those of known taxa using Bergey's

Manual of Determinative Bacteriology by Holt et al. (1994) and the scheme of Cheesbrough (2004). The cultural characteristics of the bacteria isolated were compared with the culture characteristics presented by Dubey and Maheshwari (1999). Fungi identification was based on the macroscopic and microscopic morphology. For the microscopic morphology, a drop of ethanol was placed on a clean slide with the aid of the sterile needle, a fragment of the pure culture was transferred into the ethanol on the slide and a drop of lactophenol blue stain was added and the ethanol was allowed to evaporate (Enemuor et al., 2012; Izah and Ohimain, 2013b). Then, the slides were covered with a cover slip and viewed under the microscope. The resultant microscopic and macroscopic characteristics were compared using the scheme of Bartnett and Hunter (1972), Pepper and Gerba (2004), Benson (2002). Also the characteristics presented by Ohimain et al. (2012c), Izah and Ohimain (2013b) were used for the identification.

2.2.3 Amylase screening

About 200ml of soluble starch extract (potato) was incorporated into one liter media (Nutrient Agar and Sabouraud Dextrose Agar for amylase producing bacteria and fungi respectively). The isolated pure cultures were aseptically inoculated into the respective plate for both group of organisms and incubated inverted for 24-48 hours at 37°C and 3-5 days at 30°C. The plates were then flooded with Gram's iodine for starch hydrolysis. A clear zone on the plates exposed to Gram's iodine indicates amylase production (Senthilkumar et al., 2012; Alariya et al., 2013; Akpomie et al., 2012; Benson, 2002).

2.3 Physicochemical analysis

Determination of pH

The pH was determined in-situ by the method described by APHA (1998) and Ademoroti (1996) using pH meter (HANNA HI 9820).

Determination dissolved oxygen (DO)

The dissolved oxygen was determined in-situ using DO meter (Extech 407510A), using the methods of APHA (1998) and Ademoroti (1996).

Determination of chemical oxygen demand (COD) by titration

COD was determined by titrimetric/dichromate oxidation method as described by APHA (1998) and Ademoroti (1996). The COD was calculated using the formula:

$$\text{COD (mg/l)} = \frac{(V_b - V_a) \times M \times 16000}{\text{Vol. of sample used}}$$

where;

V_b = ml of FAS used for blank

V_a = ml of FAS used for sample

M = Molarity of FAS

Determination of biochemical oxygen demand (BOD)

The biological oxygen demand (BOD) was determined using the method described by Ademoroti (1996) and APHA (1998). The 5-day BOD was computed from difference in the DO value of day 1 and day 5 multiplied by the dilution factor.

Nitrogen determination

Total nitrogen was determined using Kjeldahl indophenols (colorimetric) method as described by Ademoroti (1996). A spectrophotometer (JENWAY 6505uv/vis) wavelength of 635nm was used and the value was obtained by extrapolation from standard calibration curve.

Determination of potassium (K)

Potassium was determined by flame photometer (Perkin-Elmer) as described by Ademoroti (1996). The spectrophotometer (JENWAY 6505uv/vis) was set at 768nm. By extrapolation the concentration of potassium are calculated using the formula:

$$K \text{ (mg/l)} = \frac{\text{concentration reading on curve} \times D}{\text{ml sample}}$$

Where; D = dilution factor

Determination of oil and grease

Oil and grease determination was carried out using gravimetric method aftersoxhlet extraction (APHA, 1998) and Ademoroti (1996). The total oil and grease is calculated using the formula:

$$\text{Oil and grease (mg/L) increase in weight of flask} = \frac{(\text{mg}) \times 1000}{\text{ml of sample}}$$

Determination of total phosphate

Phosphate was determined by vanado-molybdo-phosphoric acid colorimetric method as described by APHA (1998) and Ademoroti (1996). Absorbance values were measured at 490nm using spectrophotometer (JENWAY 6505uv/vis). By extrapolation from the standard curve, the phosphate was calculated using the formula:

$$\text{PO}_4^{3-} \text{ (mg/l)} = \frac{\text{Reading from curve} \times 1000 \times D}{\text{ml of sample}}$$

Where; D = dilution factor

Determination of heavy metals (cadmium, copper, iron, chromium)

The heavy metals were analyzed using Atomic Absorption Spectrophotometer (AAS) (APHA 301A) (model: 5100 PC, Perkin-Elmer, Boston, USA) (APHA, 1998).

Statistical analysis

Microsoft Excel was used for statistical analysis including descriptive statistics (mean, standard error and proportion).

RESULTS AND DISCUSSION

The physicochemical analysis of POME from semi-mechanized palm oil mill in Bayelsa state, Nigeria is presented in Table 1. The mean values are 6.56 (pH), 4.69 mg/l (DO), 1806.33 mg/l (COD), 382.93 mg/l (BOD), 8.18 mg/l(PO_4^{3-}), 19.64 mg/l (K), 12.87 mg/l (N), 73.07 mg/l (oil and grease), 0.03 mg/l (Cd), 2.44 mg/l (Cu), 5.62 mg/l (Fe) and 2.01 mg/l (Cr). The result of this study is close to the findings of O'Thong et al. (2008) that reported a pH of 6.25 and Ohimain et al. (2012d). Other authors reported pH of 4 – 5 (Rupani et al., 2010), 4.7 (Ahmed et al., 2003). The value of pH recorded in this study is within IFC (2007) guideline value of 6 – 9 for effluent from vegetable oil processing. The value recorded in this study shows that it is tending toward neutral unlike other authors' findings. The DO value recorded during this study is close to the values reported by Ohimain et al. (2012d). The high DO recorded may be associated with the high temperature and duration of bright sunlight, which could influence the soluble gases such as

Oxygen and Carbon dioxide in the effluent (Ohimain et al., 2012d; Manjareet al., 2010). Ohimain et al. (2013b) and Ohimain and Izah (2013b) have reported atmospheric temperature during oil palm processing in the range of 27.23 – 35.60°C. DO is an essential water quality parameter in determining the physicochemical characteristics of the POME. BOD values in this study were lower than the mean values of previous report (Ohimain et al., 2012d; Wood et al., 1979). The result of this study is 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. Like BOD, COD result obtained in this study was lower than the mean values of previous studies (Ohimain et al., 2012d; Wood et al., 1979), except that of Borja et al. (1996). The value of COD recorded in this study is higher than the IFC (2007) guideline value of 250 mg/l for effluent from vegetable oil processing. The result of oil and grease in this study was higher than values reported in previous reports (Table 1). Again, it was found to be higher than the permissible discharge limit of 50mg/l for POME (Ahmed et al., 2003). IFC (2007) have stated a lower regulatory limit of 10mg/l for effluents from vegetable oil processing. The result of potassium, phosphate and nitrogen were close to the values reported by Ohimain et al. (2012d) but far from other authors' values (Table 1). The presence of potassium in POME is an indication of its richness in nutrients. Habib et al. (1997) stated that POME has high nitrogen content. The value was found to be higher than the guideline value of 10mg/l for vegetable oil processing effluent (IFC, 2007). The result of heavy metal (chromium, zinc, iron and copper) in this study were found to be higher than previous findings apart from iron concentration (Ohimain et al., 2012c; Wood et al., 1979).

Table 1: Physicochemical properties of palm oil milling effluents

Parameters	This study (mean ± standard error)	Ohimain et al., 2012d	Wood et al., 1979	Borja et al., 1996
pH	6.56±0.05	5.213 – 6.357	-	4.4
DO, mg/l	4.69±0.00	2.567 – 4.127	-	-
COD, mg/l	1806.33±7.12	1231 – 2422	42900 88250	– 30.6
BOD, mg/l	382.93±0.89	254 – 1541	17000 26700	– -
PO ₄ ³⁻ , mg/l	8.18±0.18	5.267 – 10.433	-	-
K, mg/l	19.64±0.58	9.533 – 29.143	1281 – 1928	510
N, mg/l	12.87±0.18	7.550 – 20.653	-	365
Oil and grease, mg/l	73.07±2.90	41.667 – 98.167	-	-
Cd, mg/l	0.03±0.00	0.0040 – 0.0231	0.01 – 0.02	-
Cu, mg/l	2.44±0.02	0.6143 – 1.6093	0.8 – 1.6	1
Fe, mg/l	5.62±0.07	1.8120 – 13.8127	75 – 164	205
Cr, mg/l	2.01±0.08	0.6053 – 1.6683	0.05 – 0.43	-

Table 2 present the microbial population of POME from semi-mechanized palm oil mill in Bayelsa state, Nigeria. The total heterotrophic bacteria (THB) and total fungi (TF) were 1.3×10^5 cfu/ml and 6.9×10^3 cfu/ml respectively. The POME population (bacteria and fungi) recorded during this study is comparable to values previously reported in literature (Table 2). Awotoye *et al.* (2011) reported THB and TF of soil where raw POME are discharged into as 1.8×10^6 cfu/g and 9.5×10^2 cfu/g respectively. The values of this study were higher than the microbial population of crude palm oil. Izah and ohimain (2013b) and Okechalu et al. (2011) reported THB in the order of 10^4 cfu/ml. Izah and Ohimain (2013b) reported TF of crude palm oil in the order of 10^3 cfu/ml. The microbial populations are a reflection of the physicochemical properties. Like higher animals, microorganisms require nutrient, minerals etc to thrive. Okereke et al. (2007) also reported that the volume and acidity of wastewater influences the microbial population. Ohimain et al. (2012c) reported that the microbial population is dependent on the prevailing environmental, sanitation etc measures carried out in the processing mills.

Table 2: The microbial populations of POME

Microorganisms	This study (Mean ± standard error)	Ohimain et al., 2012c	Ugoji, 1997
THB x 10 ⁶ CFU/ml	0.13±0.07	0.74– 2.00	1.30
TF x 10 ⁴ CFU/ml	0.69±0.01	3.10 – 5.70	0.13

THB = Total Heterotrophic Bacteria; TF = Total Fungi

Tables 3 and 4 presents the identification features of bacteria (biochemical test) and fungi (microscopic and macroscopic morphology) isolated from POME. The bacteria isolates include *Micrococcus* species, *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, while the fungi isolates are *Aspergillus niger*, *Aspergillus fumigatus*, *Candida* species, *Fusarium* species, *Mucor* species and *Penicillium* species. The frequency of occurrence of bacterial isolates showed that *Micrococcus* species and *Bacillus* species with 34% and 31% respectively dominated while *Staphylococcus aureus* and *Pseudomonas* species with 13% and 22% respectively are the least isolated microorganisms from POME in semi-mechanized palm oil processing mill in Bayelsa state, Nigeria (Fig.2). However in fungal isolates, *Penicillium* species, *Candida* species, *Aspergillus fumigates* and *Aspergillus niger* dominated the samples with 23%, 21%, 20% and 18% respectively while *Mucor* species and *Fusarium* species were the least isolated with occurrence frequency of 11% and 7% respectively (Fig. 3). The microorganisms identified in this study are similar to species reported by other authors. Ohimain et al. (2012c) reported *Pseudomonas* species, *Serratia* species, *Bacillus* species, *Staphylococcus* species and *Corynebacterium* species while the fungi isolates were *Aspergillus niger*, *Aspergillus flavus*, *Mucor* species, *Fusarium* species and *Penicillium* species from smallholder oil palm processing mill in Nigeria. Ugoji(1997) similarly identified *E. coli*, *Flavobacterium*, *Desulfovibrio*, *Methanococcus* sp., *Bacillus subtilis*, *Pseudomonas*, *Fusarium moniliformis*, *Penicillium* sp., *Aspergillus* sp., *Botryodiplodia theobromae*, *Trichoderma viridae* and *Geotrichum cadidum* from POME. The type of microorganisms isolated from POME is comparable to the type of organisms isolated in substrate containing oil. Okechalu et al. (2011) reported *Enterobacter* species, *Bacillus* species, *Proteus* species, *Micrococcus* species, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida* species, *Mucor* species and *Penicillium* species from crude palm oil sold in Jos metropolis, North central, Nigeria. Izah and Ohimain (2013b) reported *Enterobacter* species, *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, while the fungi isolates are *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Candida* species, *Mucor* species and *Penicillium* species from crude palm oil produced by smallholder processors in Niger Delta, Nigeria. Okereke et al. (2007) isolated *Staphylococcus* species, *Bacillus* species, *Corynebacterium* species, *Actinomycetes* species, *Pseudomonas* species, *Aspergillus* species, *Candida* species, *Rhizopus* species and *Mucor* species from crude oil spill area. Ohimain et al. (2012c) reported that nearly all POME microbial isolates are able to mineralize palm oil as carbon source.

Table 3: Biochemical test of bacteria isolates of POME

Organisms	Cultural characteristics	Gram reaction	Motility	Oxidase	Catalase	Citrate	Coagulase	Urease	Indole
<i>Pseudomonas</i> species	Mucoid colonies with umbonate elevation	Negative rod	+	+	+	+	-	-	-
<i>Bacillus</i> species	Dry, flat, and irregular, with lobate margins.	Positive rod	+	+	+	+	-	-	-
<i>Staphylococcus aureus</i>	Circular, pinhead colonies which are convex with entire margins. The colonies are golden-brown to whitish in color.	Positive cocci	-	-	+	-	+	-	-
<i>Micrococcus</i> species	Circular, pinhead colonies which are convex with entire margins. Colonies produces a bright yellow, non-diffusable pigment.	positive cocci	-	+	+	-	-	-	-

(+ = positive; - negative reactions)

Table 4: Microscopic and macroscopic morphology of fungi isolates found in POME

Organisms	Type of organisms	Microscopic morphology	Macroscopic morphology
<i>Aspergillus niger</i>	Filamentous mold	Presence of septate hyphae; black long, smooth, erect conidiophores. Hyaline, dichotomously branched vesicle, round, radiate head.	Brownish-black mycelium with dark spores on the surface.
<i>Aspergillus fumigatus</i>	Filamentous mold	Presence of rough conidiophore, with uni/biseriate phialides whose vesicle is round with radiate head. Brownish sclerotia were also observed.	Presence of blue-green to yellow coloration from surface.

<i>Candida</i> species	Ovoid sphere yeast-like	Single clusters of blastoconidia which is round and elongate. Long branched pseudohyphas were also observed.	A creamy to yellowish colonies with smooth, pasty, glistening or dry, wrinkled and dull color.
<i>Penicillium</i> species	Filamentous mold	Presence of red pigment with edges surrounded by whitish margin. Also the conidiophores are branched. Septate and fruity mycelium are observed	A bluish-green filament is seen which changes to powdery greenish brown. It has brush phialospores arrangement
<i>Mucor</i> species	Filamentous mold	Presence of visible spore and short sporangiospores with non septate hyphae	A slimy colonies texture with dark pigmented spores.
<i>Fusarium</i> species	Filamentous mold	Presence of dark pigment of micro and macro conidiophores.	Presence of sickle-shaped macroconidia that is yellow to purple in colour.

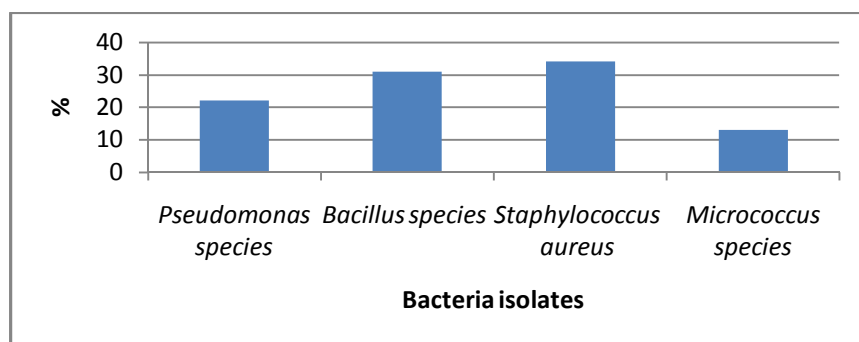


Fig. 2: Frequency of occurrence of bacteria isolated from POME

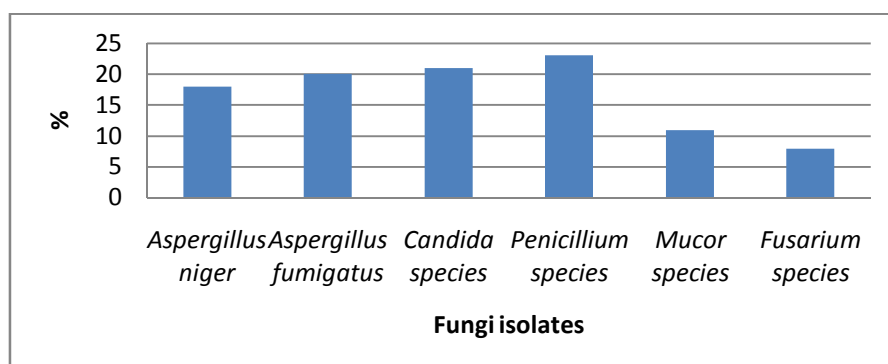


Fig. 3: Frequency of occurrence of fungi isolated from POME

POME microorganisms have been known to survive in oily waste water by producing the enzyme lipase and/ or spores. Their ability to produce spores has helped fungi to survive the anaerobic nature of POME. Ohimain et al. (2012c), Izah and Ohimain (2013b) stated that oily substrate provide a good environment for lipolytic microorganisms to flourish. Ohimain et al. (2012b) have reported that POME microorganisms could be for used for biodegradation and bioremediation in case of pollution. *Bacillus* species, *Pseudomonas* species, *Micrococcus* species and *Staphylococcus* species are lipase producing organisms. The presence of *Penicillium* species, *Fusarium* species, *Mucor* species and *micrococcus* species could be associated with their ability to survive in oily environment for long periods. *Micrococcus* species is also known for thriving under stress conditions, such as low temperature and no

food. *Micrococcus* is an opportunistic, saprotrophic pathogen. Like other hydrocarbon degraders, it can be used in detoxification or biodegradation.

The microbial isolates that produce amylase are presented in Table 5. *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, *Penicillium* species, *Fusarium* species, *Mucor* species, *Candida* species and *Aspergillus niger* were found to be amylase producing microorganisms. The result of this study is comparable to other studies where amylase were produced from diverse substrates. Arotupin (2007) reported that *Aerococcus viridens*, *Bacillus subtilis*, *Bacillus* species, *Corynebacterium manihot*, *Lactobacillus acidophilus*, *Aspergillus niger*, *Articulospora inflata*, *Geotrichum candidum*, *candida utilis*, *Saccharoyces exguusas* amylase producing microorganisms were isolated from cassava wastewater. Akpomie et al. (2012) screened bacteria from cassava peels and reported that *Bacillus subtilis*, *Bacillus megaterium*, *Corynebacterium kutseri*, *Lactobacillus fermentiare* produce α -amylase at incubation temperature of 26 to 37°C. Senthilkumar et al. (2012) reported that *Bacillus* species can produce amylase using cassava as substrate. Nwagu and Okolo (2011) isolated *Fusarium* species from soil at 50°C and determine amylase growth pattern at different temperature and found optimal amylase yield at 30°C. Ajayi et al. (2011) studied extracellular enzyme from diseased carrot and found out that bacteria (*Bacillus* species, *Leuconostoc* species, *Xanthomonas* species and *Klebsiella* species) and fungi (*Penicillium notatum*, *Mucor* species and *Fusarium* species) are able to produce amylase. Pathak and Narula (2013) studied the effect of pH on amylase production from soil mycotic flora and found out that *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* species were good amylase producers at different pH range. Varalakshmi et al. (2012) reported that *Pseudomonas* species 2 produced α -amylase from rhizosphere soil under diverse conditions of temperature and pH. Farid and Shata (2011) reported that diverse strains of *Aspergillus oryzae* and *Aspergillus awamori* produce amylase. Also, *Aspergillus niger* LS1 do not produce amylase but LS2 do. Ladokun and Adejuwon (2011) studied the incubation period of *Aspergillus fumigates* for amylase production using rice and soluble starch and they found out optimal yield on the fifth day. Alariya et al. (2013) reported that *Bacillus subtilis*, *Pseudomonas florescens*, *Escherichia coli* and *Serratia marscens* isolated from soil are able to produce amylase. Okorie and Olasupo (2013) studied growth of bacteria producing extracellular enzymes from indigenous Nigeria fermented condimentsw and reported that *Bacillus* species, *Staphylococcus* species and *Proteus* species were amylase producers. Alves et al. (2002) studied enzyme production from *Mucor* species isolated from hervivores dung and reported that *Mucor circinelloidesf. circinelloides*, *Mucor circinelloidesf. griseocyanus*, *Mucor hiemalist. hiemalis*, *Mucor racemosusf. chibinensis* and *Mucor variosporus* are good amylase producers. Kwon et al. (2007) reported that several species and strains of *Fusarium* capable of producing high amount of amylase as compared to moderate and non-producers. Khokhar et al. (2011) studied various species of *Penicillium* and *Aspergillus* for amylase production and found out that *Penicillium janthinellum*, *Penicillium melinii*, *Penicillium oxalicum*, *Penicillium velutinum*, *Penicillium waskmanii*, *Aspergillusaculeatus*, *Aspergillus ficuum*, *Aspergillus japonicas*, *Aspergillus niger*, *Aspergillus phoenix* produces amylase. Amylase production is dependent on several abiotic requirements and the substrate. Microorganisms have found application in industrial sectors particularly enzyme production. Most filamentous microbes are capable of producing extracellular enzymes in large scales. The production of amylase by these microbes could be associated with the POME richness in minerals and nutrients. Generally, POME physicochemical properties enhance the production of enzymes such as cellulase, lignin peroxidase, xylanase (Wu et al., 2009), pectinase and β -glucosidase.

Table 5: POME microbial amylase producers

Organisms	Amylase activity
Bacteria	
<i>Pseudomonas</i> species	+
<i>Bacillus</i> species	+
<i>Micrococcus</i> species	-
<i>Staphylococcus aureus</i>	+
Fungi	
<i>Aspergillus niger</i>	+
<i>Aspergillus fumigatus</i>	-
<i>Candida</i> species	+
<i>Penicillium</i> species	+
Mucor species	+
Fusarium species	+

(+ = positive; - negative reactions)

CONCLUSION

This study screened microbial amylase production from POME substrates. The microbial populations were in the order of $\times 10^5$ and $\times 10^3$ cfu/ml for THB and TF respectively. The microbial isolates were similar to those reported by other authors (Ohimain et al., 2012c; Ugoji, 1997). The physicochemical properties showed that POME is rich in mineral and nutrient and the pH is tending toward neutral contrary to previous studies. *Bacillus* species, *Pseudomonas* species, *Staphylococcus aureus*, *Penicillium* species, *Fusariums* pecies, *Mucor* species, *Candida* species and *Aspergillus niger* were found to be amylase producers. Amylase has found application in biotechnology including bioenergy and biofuel production.

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