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Research Article

Assessment of Activity of Fungal Lipase on Certain Established Parameters of Palm Oil Mill Effluent

Brendan Chibuike Kenneth[1], Amadi Benjamin Emenike[1], Agbo Esther Ujunwa[1], Iloeje Mmesomma Maryjane[1], Njoku Mary Jane[1], Obi Anasthesia Chinwendu[1], Oparaji Henry Emeka[1, *]

[1]Department of Chemical Sciences, Spiritan University, Nneochi, Nigeria

[2]Department of Biochemistry, State University of Medical and Applied Sciences, Igbo-Eno, Nigeria

Abstract

POME a bye product in palm oil processing industry is a contaminant with a high organic chemistry implication. POME is a highly polluting product, consisting of 0.7% (w/v) protein, 5% (w/v) total organic carbon, 20% total organic matter contents, 93% (w/v) water and salts the present study was carried out at a local palm oil milling center located at Umunneochi present day Abia state, Nigeria. *Aspergillus sp* was isolated from palm oil mill effluent impacted soil using culture dependent techniques. The organism was confirmed *Aspergillus* was identified using standard microbiology and biochemical techniques respectively. Lipase producing capability of the organisms was screened in an optimized culture broth in the presence of p-NPP. Yellow coloration of the broth confirms the exo-lipase secretory of the bacteria. Lipase production peaked on day 5 of the fermentation. Physicochemical properties of the palm oil mill effluent were: pH (6.0), conductivity (617 $\Omega^{-1}cm^{-1}$), DO (6.21 mg/ml), BOD_5 (4.89 mg/ml), TOC (102.34 mg/ml), TOM (125.87 mg/ml), Ca and Mg (34.15 mg/ml; 9.87 mg/ml). Lipase impact on the POME peaked at pH 5.0 after 168 hour with TOC/TOM contents of 56.2/68.15 mg/ml. DO/BOD5 at the respective conditions were 4.52 and 2.89. Decrease in the organic matter contents were seen in tangent with increase in lipase concentration. The present study showed the kinetic properties of lipase from *Aspergillus* sp. in remediation effluents such as POME as it was evident in the significant reduction in the organic matter contents of the POME.

Keywords

Pome, *Aspergillus*, Lipase, Organic Matter

1. Introduction

Palm Oil as a lipid derives its distinctive properties from the hydrocarbon nature of a major portion of the structure [22]. The molecules are composed of long chains of carbon atoms. The hydrocarbon group is modified by the presence of small numbers of more reactive polar groups [4]. The chains can exist in several bonds but have long saturated and unsaturated monocarboxylic aliphatic pure acids. Since the major portion of the lipids in the hydrocarbon, some microorganisms have been reported to degrade oil generally causing rancidity [2].

*Correspondence: Oparaji Henry Emeka (emeka.oparaji65@yahoo.com)

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POME a bye product in palm oil processing industry is a contaminant with a high organic chemistry implication [1]. POME is a highly polluting product, consisting of 0.7% (w/v) protein, 5% (w/v) total organic carbon, 20% total organic matter contents, 93% (w/v) water and salts. This organic waste from palm oil processing industries constitute to high organic oxygen quotient exertion in aquatic body in form of biochemical oxygen demand (BOD) [10, 25].

Compromised implementation of stringent standards and development of management techniques by environmentalist for the disposal of wastes into the environment has necessitated the need for the development of alternative waste treatment process. Current approaches in waste treatment have been directed towards developing enzymatic treatment systems for solid, liquid and hazardous waste is presented [3].

Lipases, (triacylglycerol acylhydrolases, E. C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. They catalyze the hydrolysis of triacylglycerols at oil-water interface [17] to release glycerol and free fatty acids [6]. Apart from other biotechnological application of lipase, ecology impact asse ssment studies have suggested great potentials of lipase in waste treatment systems [12, 20].

The enzyme ability in hydrolyase of organic matters to simple easily amenable forms of less eco-implicativeness makes them promising in environmental monitoring such as eco toxicology [9, 5].

While the palm oil industry has been perceived emphatically for its commitment toward monetary development and quick improvement, it has additionally added to environmental pollution because of the creation of huge amounts of by-products during the process of oil extraction [15]. POME contains a great amount of acidic content, temperature, Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD). At the point when it is released into streams it can defile drinking water for the human and animal community. The crude effluent contains 90-95% water and incorporates residual oil, soil particles and suspended solids. A modern oil palm plant produces about 2.5 t of effluent per ton of palm oil, or 0.5 ton of effluent per ton of new fresh fruit [19]. Palm oil plant effluent is an exceptionally a polluted substance and much exploration has beendevoted to methods for reducing its danger to the human environment [21].

2. Materials and Methods

2.1.Materials

All chemicals, prepared reagents and equipments utilized during the experimental process of the present study were of analytical grade, the equipments were calibrated and standardized; they are products of Sigma Aldrich, Merck, Trimelines companies.

2.1.1. Study Site

The present study was carried out at Toxicology laboratory of Spiritan University, Nneochi, Abia State, Nigeria.

2.1.2. Study Duration

The study period from sampling through experimental processes to result analysis was from June, 2023-Feb., 2024.

2.2. Methods

Sample Collection

Palm oil mill effluents (POME) were collected at a local palm oil milling and processing center located at Nneato, Umunneochi L. G. A of Abia at about 6.pm local time using a sterile sample container as described by APHA, 1992. The collected POME was transferred to the toxicology laboratory of Spiritan University, Nneochi for acclimatization and analysis.

2.3. Determination of Pome Physicochemical Analysis

Physicochemical properties of POME were determined as described by [1]. Parameters such as: pH, conductivity, BOD, TDS, TS, TSS, TOC and TOM were evaluated.

Lipase Production

Isolation and Identification of Strains of Aspergillus sp. from the Collected Soil Sample

Strains of the fungi (*Aspergillus* sp.) was isolated from the soil using standard microbiology (culturing/ and microscopy mounting) and biochemical (sugar fermentations, nitrogen digestions) technique as described by [11].

Screening of Aspergillus Sp for Lipase Production

Identified *Aspergillus* sp. were screened for lipase producing ability using peptone broth supplemented with 2mM p-NPP as described by [8]. The inoculated culture broth was incubated at 37 ℃ for 3 days.

Enzyme Production

Solid state fermentation technique was used for lipase production as described by [7]. Production parameters such as: Incubation period, pH, and basic macro nutrients (carbon and nitrogen) were optimized during the production process.

Assay Protocol for the Enzyme Activity

Lipase activity was determined by the colorimetric method according to [11]. This method was based on the cleavage of p-nitrophenyl palmitate (p-NPP).

Protein Determination

The total protein content of the enzyme was estimated as described by [17] using bovine serum albumin(BSA) as the standard protein. Absorbance was taken at 750nm.

Lipase Assisted Degradation of Palm Oil Mill Effluent (POME)

Degradation study on the POME was performed using the produced lipase from strains of *Aspergillus Sp*. This was out

using the gravimetric method as described by [16, 18, 24]. This procedure involves mixing equally a given concentrations of the enzyme solution (%v/v) in a sterile nutrient media optimized for the degradation study.

The media was supplemented with known concentration (%w/v) of POME and incubated for 16 days at spinning rate of 180 rpm. Afterwards extraction was done using n-Hexane to determine the weight of the oil effluent remaining after the incubation.

Work Out-plan for the Study Equation

Gravimetric method of determining percentage and rate of degradation of oil:

W2 is the weight of residual crude oil after evaporation.

W1 is the weight of empty beaker

W" weight of the conical flask and the crude oil

W"-W1= weight of residual POME (W2)

Percentage Degradation

The percentage of oil degraded= weight of POME degraded/ original weight of POME used ×100

Weight of crude oil degraded=original weight of POME used–Weight of residual crude oil obtained after evaporating the extract.

Rate of Degradation = weight of POME degraded/Time taken

Optimization of Physiologic Parameters during the Degradation Study

The following physiologic conditions were varied during the study for optimal determination of the crude oil: pH, incubation days, enzyme concentration(%v/v)and POME concentrations (%w/v).

Effect of pH

Different pH ranging from 4.5- 8.0 were prepared using respective buffer salts (sodium acetate, sodium phosphate and tris-HCl) of 1 M concentration. The mineral salt medium constituted for the POME degradation include: POME 5%, phosphate salt 0.5%, 5% enzyme solution, deionize water 200ml. These were incubated at 37 ℃ for 16 days.

Effect of Incubation Days

Mineral salt medium constituted for crude oil degradation was Incubation at different days of 0-16 days. The mineral salt medium contain: POME 5%, phosphate salt 0.5%, 5% enzyme solution, deionize water 200ml. The cultivated media were incubated at 37 ℃ and pH 5.5.

Effect of Lipase Concentrations (%v/v) onthe Degradation of Crude oil

Enzyme solution concentrations ranging from 0-20% (v/v) were varied during the degradation studies of POME. The mineral salt medium constituted include: crude oil 5%, phosphate salt 0.5%, deionized water 200 ml. The cultivated media were incubated at 37 ℃ and pH 5.5.

Effect of Crude oil Concentrations (%w/v) During the Degradation Studies

POME concentrations ranging from 0-20% (w/v) were varied during the degradation studies of POME. The mineral salt medium constituted include: enzyme suspension 5%, phosphate salt 0.5%, deionized water 200 ml. The cultivated media were incubated at 37 ℃ and pH 5.5.

3. Result and Discussion

Plate one below shows the pure mycelia growth of *Aspergillus* sp on a Potato dextrose agar prepared plates respectively. From the plates it was visible their variant filamentous colours.

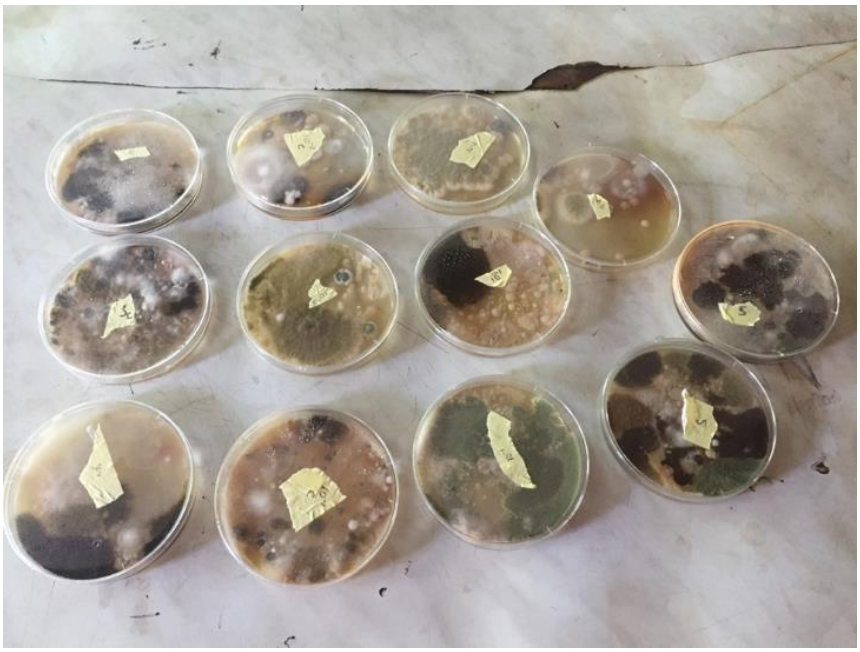

Figure 1. *Strains of Aspergillus sp. on PDA plate.*

Table1. *Physicochemical Properties of Palm Oil Mill Effluent (POME).*

PHYSIOCHEMICALFACTORS	CONTROL	POME(BEFORE REMEDIATION)
pH	7.78	5.67
CONDUCTANCE	423	610
Magnesium(Mg)(Mg/ml)	5.28	6.82
Dissolved oxygen content (mg/ml)	7.21	6.31
BOD_5 (mg.ml)	2.10	4.87
Potassium (K)(Mg/ml)	8.30	14.52
Total dissolved solid content (TDS)	258.03	372.1
Total suspended solid content(TSS)	396.07	539.55
Total solid contents (TS)	654.1	911.6
Total organic matter content (TOM)(Mg/ml)	24.88	100.7
Total organic carbon contents (Mg/ml)	20.33	81.87

N=3

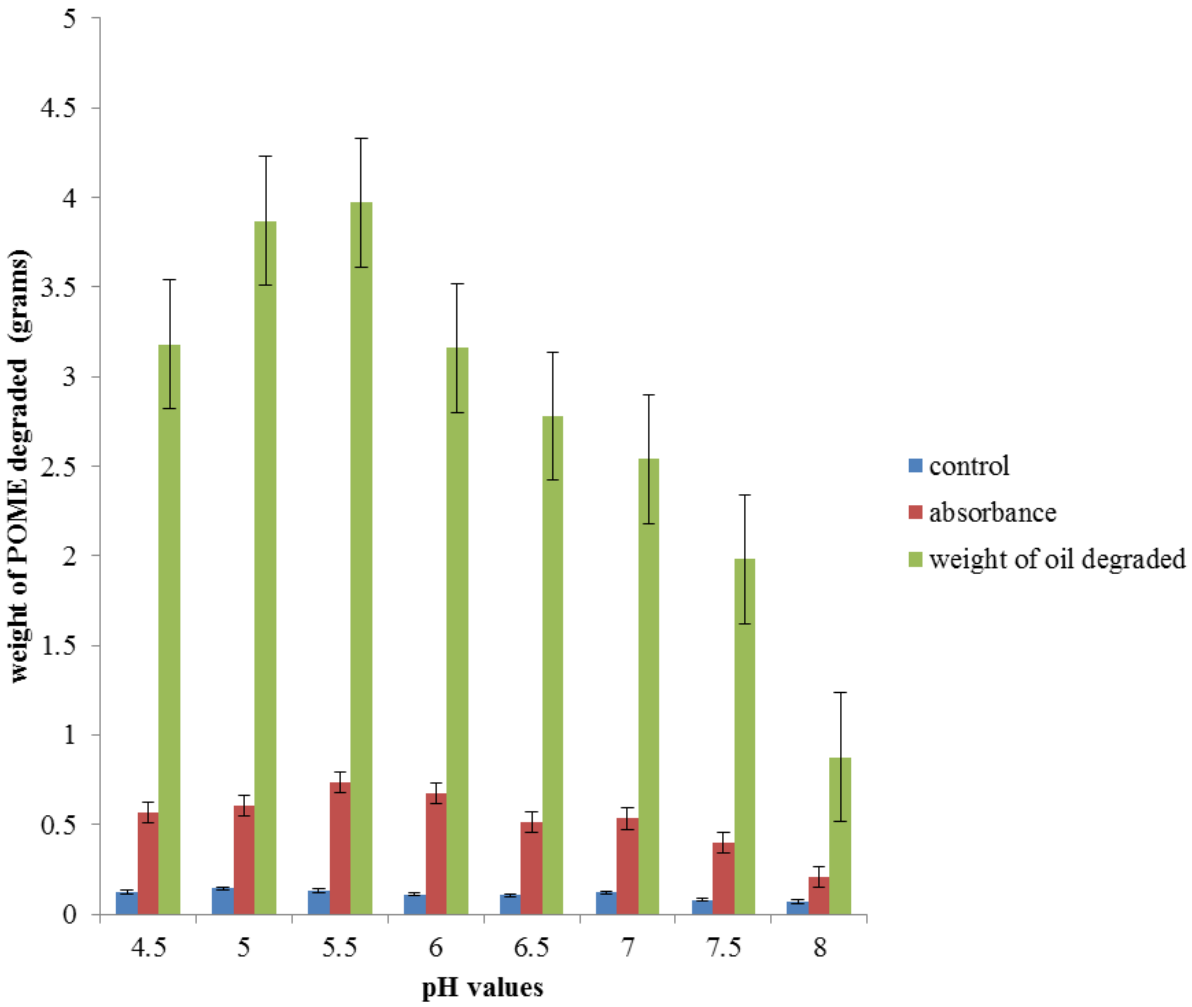

Figure 2. *Effect of pH on degradation of POME by Lipase.*

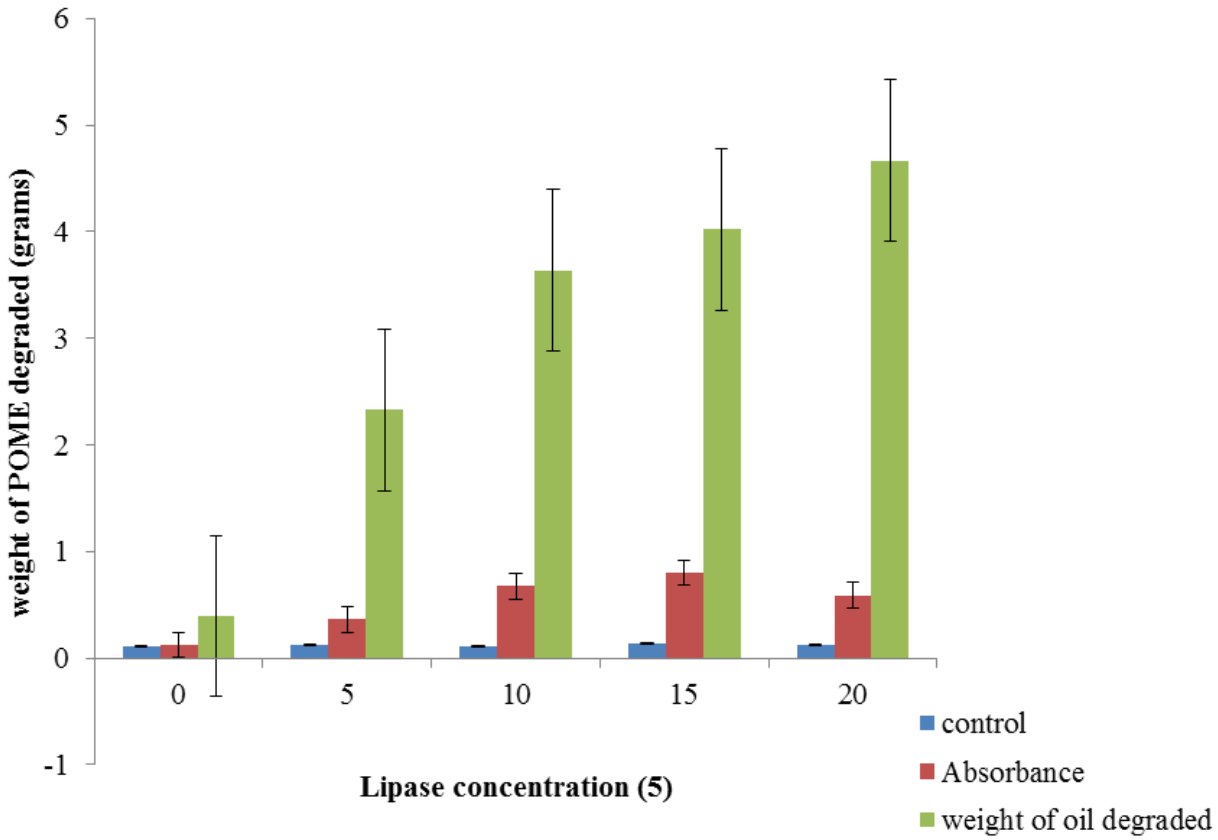

Figure 3. *Effect of Lipase concentrations on degradation of POME by lipase.*

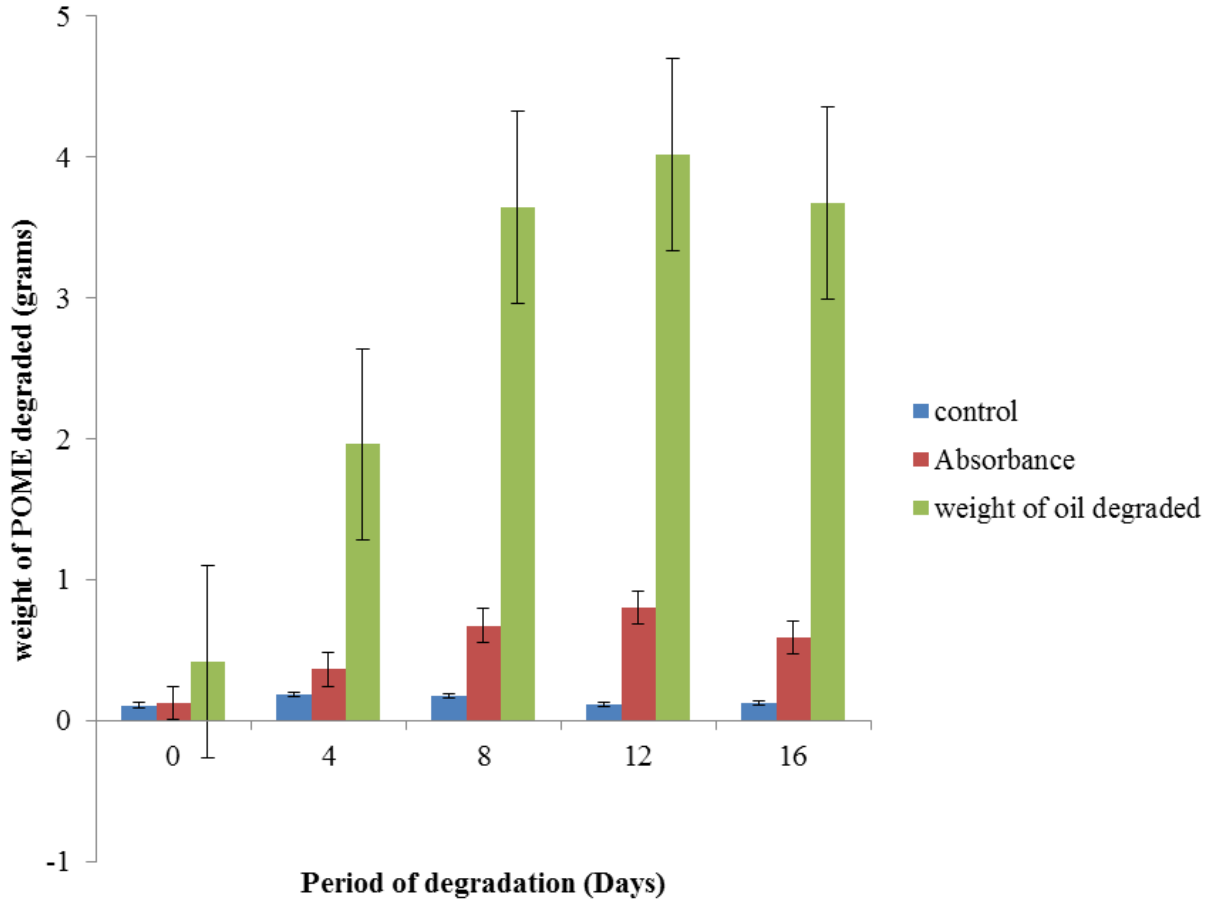

Figure 4. *Effects of incubation period on POME degradation at 10% (v/v) of the lipase incubated at pH 5.5.*

Table 2. *Physicochemical Properties of the Remediated POME.*

PHYSICOCHEMICALTEST	POME1	POME2
pH	5.67	6.5
Conductivity ($\Omega^{-1}cm^{-1}$)	610	721
TOM (mg/ml)	100.7	68.15
TOC (mg/ml)	81.87	56.2
BOD_5	4.87	2.89

N=3

4. Discussion

i. Enzymes are integral tool with vast applications in various biotechnology industries [10, 13]. Lipases, (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. They catalyze the hydrolysis of triacylglycerols at oil-water interface [23] to release glycerol and free fatty acids [10]. In contrast to esterases, lipases are activated only when adsorbed to an oil–water interface [14, 15]and do not hydrolyze dissolved substrates in the bulk fluid.

Palm oil processing is a common local technology within our locality. These processes constitutes large amounts of wastes of deleterious composites (BOD/COD) released into the surrounding. The implementation of stringent standards for the disposal of wastes into the environment has necessitated the need for the development of alternative waste treatment process. Current approaches in waste treatment have been directed towards developing enzymatic treatment systems for solid, liquid and hazardous waste is presented [18].

Physicochemical properties of the palm oil mill effluent were: pH (6.0), conductivity (617 $\Omega^{-1}cm^{-1}$), DO (6.21 mg/ml), BOD_5 (4.89 mg/ml), TOC (102.34 mg/ml), TOM (125.87 mg/ml), Ca and Mg (34.15 mg/ml; 9.87 mg/ml). Low level of pH of POME around 4-5 was reported by [21]. They went further to state that POME are characterized with high amount of organic matter content which is much evident from their oxidizable carbon content of the effluent [26, 27].

Basic morphological and biochemical screening were used to identify the isolate as*Aspergillus*. Basic morphological features of the fungus showed that strains of *Aspergillus* under an objective magnification of ×40, showed a dented spotted filamentous hyper cross-linked hyphael wall fungus with optimum growth at temperature of 30-40 ℃. These findings corroborate with that of basic manual for organisms isolations and identifications written by [18].

Lipase was produced from the isolated bacteria through solid state fermentation system after five days expressed on a formed matrix of seaweeds.

Lipase was produced from the isolated bacteria through solid state fermentation system after five days expressed on a formed matrix of seaweeds. Lipase impact on the POME peaked at pH 5.0 after 168 hour with TOC/TOM contents of 56.2/68.15 mg/ml. [15, 29] reported that most enzymatic degradation especially with esterase like lipase is mostly peaked within lower pH where higher activity of the enzyme is seen. DO/BOD5 at the respective conditions were 4.52 and 2.89. Decrease in the organic matter contents were seen in tangent with increase in lipase concentration. As described by [8] most degradation studies using biological means are optimum at lower pH and a given lengthy stipulated time. This owe to the fact of the nature of hydrocarbons and other adjourning composite of the compound [6, 28].

5. Conclusion

Lipase is a multi-purpose enzyme that has found its way in many producing industries including those of foods, clinical and environmental based research industries. The present study has shown the physicochemical properties of effluents from palm oil milling centre and their inductive impact to the environment. Application of lipase from strains of *Aspergillus* in treatment of effleunts from palm oil mill also have pave way for another plausible utilization of the enzyme in eco-monitoring and toxicological studies. Evidently before the degradation studies showed the physicochemical properties of the palm oil mill effluent and afterwards after the degradation show case the activity of the enzyme on physicochemical factors such as the total organic carbon and total organic matter, respectively.

Abbreviations

p-NPP	Para Nitrophenyl Palmitate
POME	Palm Oil Mill Effluent
BOD	Biochemical Oxygen Demand
TOM	Total Organic Matter
Toc	Total Oxidizable Carbon

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Author Contributions

Brendan Chibuike Kenneth: Conceptualization, Project administration

Amadi Benjamin Emenike: Data curation, Investigation, Resources, Writing – original draft

Agbo Esther Ujunwa: Project administration, Validation

Iloeje Mmesomma Maryjane: Validation, Resources
Njoku Mary Jane: Software, Methodology
Obi Anasthesia Chinwendu: Project administration, Validation
Oparaji Henry Emeka: Methodology, Supervision, Writing – review & editing

Conflicts of Interest

The authors of this manuscript enlisted above with the corresponding affiliations declare NO conflict of interest as regards to the present article.

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