**SCREENING OF LIPASE PRODUCING FUNGI ISOLATED FROM PALM OIL MILL EFFLUENT IMPACTED SOIL**

**ABSTRACT**

The bio-product and its application have series of setback due to selection and maintenance of strains in Nigeria today. In this study, isolation, identification and screening of lipase producing fungi from palm oil mill effluent (POME) impacted soil was carried out. The Emohua and Isiokpo palm oil mill effluent impacted soil samples were cultured and sub-cultured, identified, characterized and screened. The physicochemical analysis of the palm oil mill effluent impacted soil sample from Emohua had a pH of 6.33±0.01 while that of Isiokpo had 7.78±0.01. The electrical conductivity ranged from 60.43±0.01 -70.62±0.01 for both Emohua and Isiokpo respectively. The fungal load ranged from 3.8x104CFU/ML – 4.6x104CFU/ML for Emohua and Isiokpo respectively. The fungal genera identified were *Aspergillus* sp*, Rhizopus* sp, *Mucor* sp*, Penicillum* sp, *Fusarium* sp*, Saccharomyces* sp and *Paecilomyces* sp. The results obtained for lipase production on tributyrin media plates after 5 days of incubation showed that five isolates were positive for lipase production as indicated by diameter zone of clearance. The result further revealed that *Aspergillus* sphad the highest lipase producing ability (having a diameter zone of clearance of 19 ± 0.05 mm), followed by *Rhizopus* spand *Penicillium* sp (15 ± 0.02 mm and 14 ± 0.02 mm ) respectively. *Mucor* sp and *Fusarium* sp had 11 ± 0.01 mm and 10 ± 0.01 mm. *Paecilliomyces* sp and *Saccharomyces* sp had no zone of clearance. These results demonstrate the presence of lipase producing fungi in the palm oil mill effluent impacted soil, and can be harnessed locally for large scale production of the enzyme which is of high economic and biotechnological value in the industries today.

**Keywords: Effluent, Fungi, Lipase, Screening, Soil**

**1.0 INTRODUCTION**

Triacylglycerol acyl hydrolases (EC 3.1.1.3) also known as lipases are recognized as a group of potential industrial enzymes, responsible for catalyzing the hydrolysis or breakdown of insoluble fats and oils (triglycerides), and they can release monoglycerides, diglycerides, glycerol, and free fatty acids over an oil–water interface (Geoffry and Achur, 2018; Patel and Shah, 2020). Moreover, lipases are carboxylic acid esterases that belong to the serine hydrolase family and do not require any cofactor to catalyze the reactions (Basheer *et al*., 2011). Lipases constitute the third biggest family of digestive enzymes after proteases and carbohydrates. They are a chief group of biocatalysts in the field of biotechnology (Demera *et al*., 2019; Lima *et al*., 2019). Furthermore, these enzymes in an organic medium are also able to catalyze synthetic (formation) reactions such as esterification, interesterification, alcoholysis, aminolysis, and acidolysis in addition to hydrolysis of triglycerides (Anobom *et al*., 2014; Lima *et al*., 2019). In both aqueous and non-aqueous media, lipases have high efficiency to catalyze reactions as they contain high stability against high range of temperatures, pH, and even organic solvents. It is also known that lipases have a hydrophobic potential for their interfacial activity (Khan *et al*., 2017; Mehta *et al*., 2017; Tan *et al*., 2018; Bharathi and Rajalakshmi, 2019). Firstly, Clade Bernad, in 1856, had observed the lipase enzyme in pancreatic juice, performed the function of hydrolysis of oil and fats droplets and was capable of converting them into soluble digestible products (Jamilu *et al*., 2022). Lipases have been reported in animals, insects, and plants, as well as microorganisms such as bacteria, fungi, yeasts, and algae (Mehta *et* *al*., 2017; Sarmah *et al*., 2018; Bharathi and Rajalakshmi, 2019).

Lipases are highly diverse and mainly ubiquitous in animals, plants, and microorganisms (Bharathi and Rajalakshmi, 2019). Lipases that are specially derived from microbial sources have gained increasing attention in the industrial fields rather than those that are derived from animals and plants due to their suitable characteristic features and functional ability under highly difficult conditions and remain stable in organic solvents, chemical selectivity, enantio-selectivity, and do not need any cofactor to increase their catalytic efficiency during reactions (Bharathi and Rajalakshmi, 2019; Thapa *et al*., 2019).Among microbial-origin sources of lipases, fungi have been recognized as good producers of extracellular lipases, and processes including extraction and purification are comparatively easier than other sources of lipases (Treichel *et al*., 2010; Geoffry and Achur, 2018). Because of their versatility, fungal lipases are recognized as Potential biocatalysts in both the industrial and biotechnological sectors. Fungal lipases have applications in several industries such as leather, textile, cosmetics, biodiesel production, detergent manufacturing, medicine and pharmaceutical, pulp and paper production, dairy product formation, beverages, medical and diagnostics, biosurfactant formation, fatty acid production, and the oleochemical industry (Kaur *et al*., 2016; Geoffry and Achur, 2018; Avhad and Marchetti, 2019; Jamilu *et al*., 2022). The production of fungal lipases is largely affected by the composition of the medium, temperature, pH, inoculum volume, aeration, agitation, and several other factors. These other factors have been observed that affect the number of microbes, and several strategies have been applied that optimize the different parameters of the fermentation process by using statistical experimental designs (Kishan *et al*., 2013; Lima *et al*., 2019).

**2.0 MATERIALS AND METHODS**

**2.1 Collection of Soil Samples**

Palm oil mill effluent impacted soil samples for this study were obtained from Emohua and Isiokpo in sterile sample bottles. The samples were kept in an ice pack and immediately transported to laboratory for analyses.



**Plate 1: Sample collection point from Emohua**

## 2.2 Physicochemical analysis of the palm oil impacted soil

## 2.2.1 pH

The electrochemical method was used in accordance with the procedure detailed in (IITA, 1979): To calibrate the pH meter at 7.0, two grams (2g) of phosphate power seeded in 20ml of deionized water was used as a buffer; the reference electrode was lowered into the liquid sample. The mean of two readings from the samples were calculated (IITA, 1979).

## 2.2.2 Determination of Alkalinity of Soil (APHA 2320B)

To determine the soil's alkalinity, a chemical method based on the Dewis and Freitas (1970) procedure was used. Pipette 10 ml of hydrochloric acid into 50 ml Erlenmeyer flask. Weigh the Erlenmeyer flask with cork stopper. Transfer 1 to 10 gm of soil to the flask. After effervescence has largely subsided make the stopper loose and swirl the flask. The reaction is usually complete in two hours. Displace the carbon dioxide in the flask with air and weigh the flask with stopper.

Calculations

Weight of CO2 lost = (Initial wt. of flask + acid + soil) – (Final wt. of flask + acid + soil)

## 2.2.3 Electrical Conductivity (APHA 2510B)

The electrometric method was used in accordance with the WinLab conductivity device. After calibrating the meter with standard solutions, the probe was lowered into the sample and measurements were taken once the reading stabilized. The reading was expressed in µS/cm.

## 2.2.4 Determination of Salinity (APHA 2520B)

The sample's salinity was determined using a refractometric technique (HRN -2N ATAGO Japan). The reference sample and water were used to obtain the readings.

## 2.2.5 Determination of Phosphate (APHA 4500PC)

A quarter (25ml) of the sample was decanted into a 250ml conical flask and the volume was reconstituted to the final volume. 0.5g potassium persulphate and 2ml of 2M tetraoxosulphate (VI) acid were added to five millilitres of the mixture in a 250ml conical flask. The mixture was heated until it reached the digest condition. One drop of phenolphithalein indicator was added, and the solution was neutralized with 1.0 N sodium hydroxide base. Five millilitres (5 ml) of standard solution was added and brought to the final concentration before homogenization and spectrometry at a wavelength of 650nm (spectronic 20, Genesys, Thermos, USA). The relationship was used to calculate the phosphate concentration.

## 2.2.6 Determination of Ammonia in Soils (Method APHA 4500-NH3-B)

The concentration of exchangeable NH4 or Ammonia nitrogen in soils was determined using the boric acid titration method with 5% sulphuric acid. 10-g triplicate samples of the < 2-mm fraction of the air-dry soils were extracted with 100 ml of 2M KC1 for 1 hour in a wrist action shaker; the suspension was filtered, and the entire filtrate steam distilled with 0.5 g dry, carbonate-free MgO for 6 to 7 min, at a rate of 5-6 ml/min. The time of distillation and amount of MgO used were those standardized in a preliminary study. The ammonia was absorbed in 25 ml of 2% boric acid and titrated with 0.02/0.05 N H2SO4.

Calculation: Exchangeable NH4-N% in soil = (Vs- VB) \* S \* 0.014 \* 100/W = Z

Exchangeable NH4+N (ppm) = Z \* 104

Where, Vs= vol. of H2SO4 required for sample titration

 VB = vol. of H2SO4 required for blank titration

 S = strength of H2SO4 W = wt of oven dry soil.

## 2.2.7 Determination of Sulphate in Soil (Turbidimetric Method)

The sulphate concentration was determined using a turbidimetric technique with 2.0g barium chloride. 0.5 N ammonium acetate solution with pH 4.2 (diluted with HCL) is added ti a 10-gram representative sample of air-dried soil to form a solution of 1:5 by weight. The mixture is boiled for 5 minutes and filtered through Whatman No. 40 filter paper until a clear filtrate is obtained 10ml of extracted solution is diluted to 40ml with distilled water. 0.2 gram of barium chloride is added, and the mixture is diluted to 50ml. The solution is stirred for one (1) minute and the turbidity measured using a spectrophotometer at 30 second intervals for 4 minutes. The maximum reading is considered as the turbidity and the value is compared with a standard curve to determine the sulphate ion concentration.

**2.3. Microbiological analysis of the soil sample**

## 2.3.1 Enrichment of samples

Ten gram samples (from each site) were suspended in 250 mL Erlenmeyer flask containing 100mL sterile physiological saline. This was followed by constant and vigorous stirring for 30 mins at 120 rpm to dislodge soil clumps before allowing settling. The supernatant was decanted and a 10–fold serial dilution made from it. Serial dilutions were prepared and 0.1 ml from 10-2 and 10-4 dilution was cultivated on Potato Dextrose Agar (PDA) and incubated at 27oC for 5 days. Grown fungal colonies were then purified on PDA plates.

**2.4 Total fungal count**

The total fungal count were determined by dissolving 0.1 ml of theenrichment broth and diluted in a 10-fold serialdilution, itwas then plated on Potato dextrose agar (PDA) fortified with 0.1% lactic acid to inhibit bacterial contaminants. The supernatant was decanted and a 10–fold serial dilution made from it. Serial dilutions were prepared and 0.1 ml from 10-2 and 10-3 dilution was cultivated on PDA and incubated at 27oC for 5 days. Grown fungal colonies were then purified on PDA plates.

**2.5 Characterization and Identification of Fungal Isolate**

**2.5.1 Fungal Macroscopy**

This was done to determine the colonial characteristics of the isolates on Potato Dextrose Agar (PDA) plates. The isolates were aseptically inoculated on freshly prepared PDA plates and incubated at 27°C for 3 - 5 days. After incubation, properties like the presence of aerial and substrate mycelia with their colour, shape of the colony, form and diameter were observed.

**2.5.2 Fungal Microscopy**

Microscopically, features like cell shape, type of hypha, presence of spores and spore arrangement were also observed. The cells were first stained prior to microscopic examination. Filamentous fungal isolates were aseptically cut and placed on a clean slide, flooded with the lacto phenol cotton blue dye and fixed using a cover slip (wet mounting). The slide was then viewed with the x 40 objective lens.

**2.5.3 Fungal Characterization**

The fungal isolates obtained from the soil were characterized on the basis of their macroscopic and microscopic properties alone (Jamilu *et al*., 2022).

**2.5.4 Fungal Microscopy**

This was done to determine the colonial characteristics of the isolates on Potato Dextrose Agar (PDA) plates. The isolates were aseptically inoculated on freshly prepared PDA plates and incubated at 28°C for 3 - 5 days. After incubation, properties like the presence of aerial and substrate mycelia with their colour, shape of the colony, form and diameter were observed.

Microscopically, features like cell shape, type of hypha, presence of spores and spore arrangement were also observed. The cells were first stained prior to microscopic examination. Filamentous fungal isolates were aseptically cut and placed on a clean slide, flooded with the lacto phenol cotton blue dye and fixed using a cover slip (wet mounting). The slide was then viewed with the x 40 objective lens.

**2.6. Screening of isolates for lipase production**

Screening of lipase producing fungi was done using tributyrin as a substrate on agar plates. For this purpose tributyrin agar (HiMedia) composed of: Peptone: 5.0gm; Yeast extract: 3.0gm; Agar agar: 15.0gm; Tributyrin (Glycerol Tributyrate): 10.0ml; Distilled water: 990 ml; pH: 7.5 was used. The formation of the clear zone around the colonies indicates the production of lipase. All the isolated fungal culture were inoculated on the TBA plates and incubated at 27°C up to 5days. The formation of opaque zones around the colonies is an indication of lipase production by the microorganisms.

**2.7 Statistical Analysis**

Statistical analysis using statistical package for social science (SPSS) statistical software in version 21.0 to carry out Analysis of variance (ANOVA) at p< 0.05 level of significance.

**3.0 RESULTS**

## 3.1: The Physicochemical Profile of the Palm oil mill effluent impacted soil

Table 1 shows the physicochemical profile of the palm oil mill effluent impacted soil in Emohua and Ikwerre Local Government, Rivers State, Nigeria. The pH of Emohua palm oil mill site had 6.33±0.01 while that of the Isiokpo palm oil mill site had 7.78±0.01. The temperature of the soil samples were 26.2±0.01 and 26.5±0.01 (oC) for the Emohua and Isiokpo palm oil processing sites respectively. The Electrical conductivity for Emohua soil sample was 60.43±0.01 µS/cm while that of the Isiokpo soil was 70.62±0.01 µS/cm. The salinity of the Emohua soil sample was 5.149±0.01 mg/Kg while for the Isiokpo was 5.133±0.01mg/Kg. The Alkalinity of the Emohua soil sample was 26.17±0.01mg/Kg while that of the Isiokpo soil was 80.22±0.01 mg/Kg respectively. Phosphate content of the soil samples were 3.48±0.01mg/kg and 3.86±0.01mg/kg for Emohua and Isiokpo palm oil mill effluent impacted sites respectively. Ammonia content of the Emohua and Isiokpo soil sample were 0.066±0.01 and 0.072±0.01 mg/Kg. The total organic carbon of the Emohua and Isiokpo palm oil mill effluent impacted sites were 2.084±0.01 and 2.168±0.01 mg/Kg respectively. The Sulphates of the Emohua and Isiokpo palm oil mill effluent impacted sites were 241.74±0.06 and 159.38±0.01mg/Kg.

**Table 1: Physicochemical Composition of Soil samples**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Emohua Palm oil Mill Impacted soil |  | Isiokpo Palm oil Mill Impacted soil |  |
| pH | 6.33±0.01a |  | 7.78±0.01b |  |
| Temperature (oC) | 26.2±0.01 a |  | 26.5±0.01 a |  |
| Conductivity (µS/cm) | 60.43±0.01 a |  | 70.62±0.01 b |  |
| Salinity (mg/kg) | 5.149±0.01 a |  | 5.133±0.01 a |  |
| Alkalinity (mg/kg) | 26.17±0.01 a |  | 80.22±0.01c |  |
| Phosphate (mg/kg) | 3.48±0.01 a |  | 3.86±0.01 b |  |
| Ammonia (mg/kg) | 0.066±0.01 b |  | 0.072±0.01 c |  |
| TOC (%) | 2.084±0.01 a |  | 2.168±0.01 b |  |
| Sulphates (mg/kg) | 241.74±0.06 a |  | 159.38±0.01 b |  |
|  |  |  |  |  |

Data presented as Mean ± Standard Deviation; Similar superscripts in a column imply there was no significant difference, those with different superscripts are significant at p-value <0.05

**3.2 The Fungal Count from Emohua and Isiokpo palm oil mill effluent impacted soil**

Table 2 shows the fungal count obtained from Emohua and Isiokpo palm oil mill effluent impacted soil during the study. The Emohua palm oil mill effluent impacted soil had a total fungal count of 4.6× 104 CFU/ml while the Isikpo palm oil mill effluent impacted soil sample had a total fungal count of 3.8X104CFU/ml.

**Table 2: Fungal population of palm oil mill effluent impacted soil**

|  |  |  |  |
| --- | --- | --- | --- |
|  **Sample** |  |  |  **FC(Cfu/ml)** |
|  Emohua (A) |   |   |  4.6X104 |
|  Isiokpo (B) |  |  |  3.8X104 |
|  |   |   |  |

Key: FC= Fungal Count

**3.3 Characterization of Fungal Isolates from Emohua and Isiokpo palm oil mill effluent impacted soil**

Tables 3a and 3b show the characterization of fungal flora isolated from Emohua and Isiokpo palm oil mill effluent impacted soil sample. The macroscopy and microscopy of the fungi on the potato dextrose agar, suggest that a distinct preliminary relationship between the morphological and molecular characterization. The fungal flora of Emohua and Isiokpo palm oil mill effluent impacted soil contained a wide variety of fungal flora like the *Aspergillus* sp*, Paecilomyces* sp, *Penicillium* sp*, Rhizopus* sp*, Saccharomyces* sp*, Fusarium* sp *and Mucor* sp.

**Table 3a: Characterization of fungal isolates from Emohua palm oil mill effluent impacted soil**

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate Code** | **Macroscopy** | **Microscopy** | **Probable isolate** |
| ES1 | Black, velvety with a yellow halo | Conidia-borne and long conidiophore | *Aspergillus* sp. |
| ES2 | Colony with a yellow-brown aerial mycelium | Dense conidiophores with branched cells | *Paecilomyces* sp. |
| ES3 | Colony with a yellowish-green aerial mycelium | Dense conidiophores with branched cells | *Penicillum* sp. |
| ES4 | Colony with a yellow-brown aerial mycelium | Dense conidiophores with branched cells | *Paecilomyces* sp |
| ES5 | Black, velvety with a yellow halo | Conidia-borne and long conidiophore | *Aspergillus* sp. |
| ES6 | White coloured colonies with smooth surface | Yeast-like cells with no hyphae or pseudohyphae | *Saccharomyces* sp. |
| ES7 | Colony with a yellow-brown aerial mycelium | Dense conidiophores with branched cells | *Paecilomyces* sp |
| ES8 | Whitish mycelium | Branched sporangiophore with spored sporangium .absence of rhizoids | *Mucor* sp. |
| ES9 | Whitish-grey dense growth covering the plate. | Cornidia borne with a thread-like sporangiophores | *Rhizopus* sp |

**Table 3b: Characterization of fungal isolates from Isiokpo palm oil mill effluent impacted soil**

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate Code** | **Macroscopy** | **Microscopy** | **Probable isolate** |
| IS1 | Whitish mycelium | Branched sporangiophore with spored sporangium .absence of rhizoids | *Mucor* sp. |
| IS2 | Colony with a yellow-brown aerial mycelium | Dense conidiophores with branched cells | *Paecilomyces* sp. |
| IS3 | Colony with a yellowish-green aerial mycelium | Dense conidiophores with branched cells | *Penicillum* sp. |
| IS4 | Whitish grey mycelium | Branched sporangiophore with spored sporangium .absence of rhizoids | *Mucor* sp. |
| IS5 | Whitish and cottony colony with a pink pigments behind | Branched conidiophores with smooth conidia in chains or pairs | *Fusarium* sp. |
| IS6 | Colony with a yellow-brown aerial mycelium | Dense conidiophores with branched cells | *Paecilomyces* sp. |
| IS7 | White coloured colonies with smooth surface | Yeast-like cells with no hyphae or pseudohyphae | *Saccharomyces* sp. |

**3.4 The Lipase production potential screening of fungal isolates obtained from palm oil mill effluent impacted soil**

Table.4 shows the lipase production potential screening of fungal isolates obtained from palm oil mill effluent impacted soil during the study. The *Aspergillus* sp. had the highest lipase producing ability (having a diameter zone of clearance of 19 ± 0.05 mm), followed by *Rhizopus* sp and *Penicillum* sp (having 15 ± 0.02 mm and 14 ± 0.02 mm) respectively. The *Mucor* sp and *Fusarium* sp had11 ± 0.01 mm and 10 ± 0.01 mm respectively. *Paeciliomyces* sp and *Saccharomyces* sp had no zone of clearance.

**Table 4: Lipase production potential screening of fungal isolates obtained from palm oil mill impacted effluent soil**

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolates code** |  **Fungal isolates**  |  | **Zone of Clearance (mm)** |
| ES1, ES5 |  *Aspergillus* sp. |  | 19±0.05 |
| ES2,ES4,ES7,IS2,IS6 |  *Paeciliomyces* sp. |  |  0±0.00 |
| ES3,IS3 |  *Penicillum* sp |  | 14±0.02 |
| ES6, IS7 |  *Saccharomyces* sp |   |  0±0.00 |
| ES8, IS1, IS4ES9IS5 |  *Mucor* sp *Rhizopus* sp *Fusarium* sp |  | 11±0.0115±0.0210±0.01 |

**DISCUSSION**

Palm oil mills in the southern part of Nigeria has remained a mainstay for a number of economic benefits. The activities from the milling have been reported in both cottage and commercial scale in major states of Nigeria. The need for proper treatment of POME have been advocated by other researchers (Nwachukwu *et al*., 2018; Okute and Isu, 2007). There have been concerns that indiscriminate discharge of POME may impact on the physicochemical attributes such as the total hydrocarbon content, and level of dissolved oxygen as reported by Ohimain *et al*. (2012). The impact of these industrial activities on the soil have been reported to have a number of negative effects ranging from aesthetic loss to tainting and leaching of nutrients. The account of Iyakndue *et al.* (2017) suggests a marked change in the physicochemical and nutrient availability for the soil pre-exposed to palm oil mill effluent. Chikwendu and Ogbonna (2018) reported a high level of soil radicals. Their study also documented a change in their microbial flora. The pH of the impacted soil ranged from 6.33±0.01 for Emohua while pH 7.78±0.01 was recorded for Isiokpo soil. The pH of any soil has been credited and correlated with the microbial activities, nutrient uptake, diversity and bioavailability. This ranges observed was in tandem with the report of Iyakndue *et al.* (2017) whose investigation reported pH 7.48. There was a steady decline in the pH values of the polluted soil making them slightly acidic. This observation was similar to the report of Iwuagwu and Ugwuanyi (2014) whose study reported a similar decline in the pH of the POME-impacted soil. In a related study, the report of Nnaji *et al*. (2016) also reported that there was a steady decline in the pH of an impacted soil, but also observed that the steadily returned to alkaline condition from the acidic condition caused by the palm oil mill effluent. POME-impacted soil has been widely reported to be acidic and could create a number of acidic soil challenges (Awotoye *et al*., 2011). The total fungi counts (in logarithm) are 3.8x104cfu/ml - 4.6x104cfu/ml for the Emohua and Isiokpo palm oil mill effluent impacted soil respectively. The values of the microorganism slightly agrees with the earlier report of Frączek *et al*., (2014) whom reported high concentration of fungal load about 5.6 X 104-5.0 X 104Cfu/g reported by the latter which agrees with the findings of this research. The fungal counts contribute in biotechnological application. The fungal flora isolated from the soil indicate the rate of invasiveness of the run offs from these palm oil mill effluent. Emohua sample had *Aspergillus* sp, *paecilomyces* sp, *Penicillium* sp*,* *Saccharomyces* sp, *Mucor* spand *Rhizopus* sp. The sample from the Isiokpo indicate the dominance of *Saccharomyces* sp, *paecilomyces* sp, *Fusarium* sp, *Penicillium* sp and *Mucor* sp. This result agrees with the report of Williams *et al*., (2021) which have similar results with this study where *Mucor* sp had the lowest frequency of occurrence. This also agrees with the report of Uzoigwe and Agwa, (2012) that reported the prevalence of *Aspergillus* sp. Most of the fungal isolates identified in the soil have been attributed with severe cases of industrial benefit while some other reports have applied some microbial groups from soil as a reservoir of biotechnologically diverse fungi. Among all the seven fungal species isolated, five organism which are *Aspergillus* sp, *Penicillium* sp*,* *Mucor* sp*, Rhizopus* sp and *Fusarium* sp were found to produce varying amount of lipase. The appearance of a zone of clearance was used for the indication for lipase production. The diameter of the zone of clearance of the different isolates showed that *Aspergillus* sp had the highest, followed by *Rhizopus* spand *Penicillium* sp, then *Mucor* sp and *Fusarium* sp. The importance of lipases in various industries cannot be overstated, and their use is growing in a number of areas. According to Mueller *et al* (2016), extensive and ongoing screening for new microorganisms and their lipolytic enzyme will open new, simple synthetic routes and as a result, new and faster ways to apply lipases to improving human life, including solving biotechnological and environmental inconvenience.

**4. CONCLUSION**

The *Aspergillus* sp, *Penicillium* sp*,* *Mucor* spand *Rhizopus* sp and *Fusarium* sp has some industrial uses and biotechnological application such as production of metabolites like bioproduct, laundry and dish-washing detergents, assists in bioremediation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

Anobom, C. D., Pinheiro, A. S., De-Andrade, R. A., Aguieiras, E. C., Andrade,G. C., Moura, M. V., *et al*. (2014). From structure to catalysis: Recent developments in the biotechnological applications of lipases. *Biomed Res. Int.* 2014, 684506. doi: [10.1155/2014/684506](https://doi.org/10.1155/2014/684506)

Avhad, M. R., and Marchetti, J. M. (2019). “Uses of enzymes for biodiesel production,” in *Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts* (Sawston: Woodhead Publishing), 135–152. doi: [10.1016/B978-0-12-817941-3.00007-3](https://doi.org/10.1016/B978-0-12-817941-3.00007-3)

Awotoye, O. O., Dada, A. C., & Arawomo, G. A. O. (2011).Impact of palm oil processing effluent discharge on the quality of receiving soil and river in South Western Nigeria.*Journal of Applied Sciences Research*, *7*(2), 111-118.

Basheer, S. M., Chellappan, S., Beena, P. S., Sukumaran, R. K., Elyas, K. K., and Chandrasekaran, M. (2011). Lipase from marine *Aspergillus awamori* BTMFW032: Production, partial purification and application in oil effluent treatment. *New Biotechnol*. 28, 627–638. doi: [10.1016/j.nbt.2011.04.007](https://doi.org/10.1016/j.nbt.2011.04.007)

Bharathi, D., and Rajalakshmi, G. (2019). Microbial lipases: An overview of screening, production and purification. *Biocatal. Agric. Biotechnol*. 22, 101368. doi: [10.1016/j.bcab.2019.101368](https://doi.org/10.1016/j.bcab.2019.101368)

Chikwendu, M. U., & Ogbonna, A. N. (2018).Impact of palm oil mill effluent on the soil in Upkom-Bende forest reserve, Abia state, Nigeria.*Journal of Research in Forestry, Wildlife and Environment*, *10*(3), 55-61.

Demera, L. L., Barahona, P. P., and Barriga, E. J. C. (2019). Production, extraction and characterization of lipases from the antarctic yeast *Guehomyces pullulans*. *Am. J. Biochem. Biotechnol*. 15, 75–82. doi: [10.3844/ajbbsp.2019.75.82](https://doi.org/10.3844/ajbbsp.2019.75.82)

Geoffry, K., and Achur, R. N. (2018). Screening and production of lipase from fungal organisms. *Biocatal. Agric. Biotechnol*. 14, 241–253. doi: [10.1016/j.bcab.2018.03.009](https://doi.org/10.1016/j.bcab.2018.03.009)

Iwuagwu, J. O., and J. O. Ugwuanyi (2014). "Treatment and valorization of palm oil mill effluent through production of food grade yeast biomass." *Journal of Waste Management*.

Iyakndue, M. L., Brooks, A. A., Unimke, A. A., & Agbo, B. E. (2017). Effects of palm oil mill effluent on soil microflora and fertility in Calabar–Nigeria. *Asian Journal of Biology*, 1-11.

Jamilu, H., Ibrahim, A. H., and Abdullahi, S. Z. (2022). Isolation, optimization and characterization of lipase producing bacteria from abbatoir soil. *Int. J. Adv. Sci. Technol*. 3, 2708–7972 doi: [10.51542/ijscia.v3i1.9](https://doi.org/10.51542/ijscia.v3i1.9)

Kaur, G., Singh, A., Sharma, R., Sharma, V., Verma, S., and Sharma, P. K. (2016). Cloning, expression, purification and characterization of lipase from *Bacillus licheniformis*, isolated from hot spring of Himachal Pradesh, India. *3 Biotech* 6, 1–10. doi: [10.1007/s13205-016-0369-y](https://doi.org/10.1007/s13205-016-0369-y)

Khan, F. I., Lan, D., Durrani, R., Huan, W., Zhao, Z., and Wang, Y. (2017). The lid domain in lipases: Structural and functional determinant of enzymatic properties. *Front. Bioeng. Biotechnol*. 5, 16. doi: [10.3389/fbioe.2017.00016](https://doi.org/10.3389/fbioe.2017.00016)

Kishan, G., Gopalakannan, P., Muthukumaran, C., Muthukumaresan, K. T., Kumar, M. D., and Tamilarasan, K. (2013). Statistical optimization of critical medium components for lipase production from *Yarrowia lipolytica* (MTCC 35). *J. Genet. Eng. Biotechnol*. 11, 111–116. doi: [10.1016/j.jgeb.2013.06.001](https://doi.org/10.1016/j.jgeb.2013.06.001)

Lima, L. G. R., Gonçalves, M. M. M., Couri, S., Melo, V. F., Sant’Ana, G. C. F., and Costa, A. C. A. D. (2019). Lipase production by *Aspergillus niger* C by submerged fermentation Braz Arch Biol Technol., 62. doi: [10.1590/1678-4324-2019180113](https://doi.org/10.1590/1678-4324-2019180113)

Mehta, A., Bodh, U., and Gupta, R. (2017). Fungal lipases: A review. *J. Biotech Res*.8, 58.

Mueller S, Ahmed KZ, Singh A, Davis AK, Mukhopadyay S, Swaminathan M, Mano Y, Wang Y, Wong J, Bharathi S, Moghadam HF (2016). Design of high efficiency integrated voltage regulators with embedded magnetic core inductors. IEEE 66th Electronic Components and Technology Conference (ECTC); 566-573. IEEE.

Nnaji, J. C., Okoye, J. A., & Omotugba, S. K. (2016).Soil quality in the vicinity of palm oil mills in Umuahia, Nigeria.*International Research Journal of Chemistry and Chemical Sciences*, *3*(1), 029-032.

Nwachukwu J.N., Njoku, O., Agu, C., Okonkwo, C. and Obidiegwu, J. (2018) Impact of Palm oil mill effluent contamination on soil enzyme activities and physicochemical properties.*Research Journal of Environmental Toxicology*. 12: 34-41.

Ohimain, E. I., Seiyaboh, E. I., Izah, S. C., Oghenegueke, V., & Perewarebo, T. (2012). Some selected physico-chemical and heavy metal properties of palm oil mill effluents. *Greener Journal of Physical Sciences*, *2*(4), 131-137.

Okwute, O. L., & Isu, N. R. (2007).Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State. *African Journal of Biotechnology*, *6*(2).

Patel, G. B., and Shah, K. R. (2020). Isolation, screening and identification of Lipase producing fungi from cotton seed soapstock. *Indian J. Sci. Technol*. 13, 3762–3771. doi: [10.17485/IJST/v13i36.1099](https://doi.org/10.17485/IJST/v13i36.1099)

Sarmah, N., Revathi, D., Sheelu, G., Yamuna Rani, K., Sridhar, S., Mehtab, V., *et al*. (2018). Recent advances on sources and industrial applications of lipases. *Biotechnol. Prog*. 34, 5–28. doi: [10.1002/btpr.2581](https://doi.org/10.1002/btpr.2581)

Tan, J. S., Abbasiliasi, S., Ariff, A. B., Ng, H. S., Bakar, M. H. A., and Chow, Y. H. (2018). Extractive purification of recombinant thermostable lipase from fermentation broth of *Escherichia coli* using an aqueous polyethylene glycol impregnated resin system. *3 Biotech* 8, 1–7. doi: [10.1007/s13205-018-1295-y](https://doi.org/10.1007/s13205-018-1295-y)

Thapa, S., Li, H., OHair, J., Bhatti, S., Chen, F. C., Nasr, K. A., *et al*. (2019). Biochemical characteristics of microbial enzymes and their significance from industrial perspectives. *Mol. Biotechnol*. 61, 579–601. doi: [10.1007/s12033-019-00187-1](https://doi.org/10.1007/s12033-019-00187-1)

Treichel, H., de Oliveira, D., Mazutti, M. A., Di Luccio, M., and Oliveira, J. V. (2010). A review on microbial lipases production. *Food Bioproc. Tech.* 3, 182–196. doi: [10.1007/s11947-009-0202-2](https://doi.org/10.1007/s11947-009-0202-2)