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# Improvement in Oxygen Demand Capacities of Palm Oil Mill Effluent from Ujiogba Edo State Using Some Selected Bacterial and Fungal Isolates

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**Abstract**

Raw palm oil mill effluent contains a high quantity of organic matter that confers a high amount of biological oxygen demand (BOD) and chemical oxygen demand (COD) on it. Untreated palm oil mill effluent (POME) causes pollution of the environment affecting microorganisms, plants, and humans. The study investigated the bio-treatment capabilities of *Pseudomonas* sp., *Bacillus*, *Penicillium,* and *Aspergillus* species on POME from three oil palm milling sites. These sites have been receiving POME for over a decade at Ujiogba, Edo State, Nigeria. *Bacillus* sp. and *Pseudomonas* sp. were inoculated into the bio-treatment and reduced BOD by 60.79 % and 58.65 %, respectively, whereas the consortium of both isolates reduced BOD by 72.37 %. *Aspergillus* sp. had a percentage reduction value of 65.75%. *Penicillium* sp. had 70.19% while the consortium of *Aspergillus* sp. and *Penicillium* sp. had a percentage reduction of 72.89%. For COD, *Bacillus* sp. and *Pseudomonas* sp. had a reduction of 70.67% and 72.80% respectively, while their consortium had a reduction of 75.31%. *Aspergillus* sp. recorded 50.26%, while *Penicillium* sp. gave a 73.79% reduction. The results showed that the selected isolates, particularly the consortium, showed improved oxygen capabilities of POME thereby increasing its biodegradation and, subsequently, leading to reduced pollution and enhanced environmental sustainability.



**Keywords:** Bio-treatment, Environmental sustainability, Oxygen demand, *Pseudomonas* sp., *Penicillium* sp.

## 1. Introduction

Untreated Palm oil mill effluent (POME) contaminants are more than 100 times compared to household sewage (Lee *et al.,* 2018). The oil palm business remains a main component of the agro-allied industries in Nigeria, and POME is a waste produced in the course of palm oil production. The release of fresh POME directly into soils influences the nearby bodies of water during runoff, which leads to increased acidity and eutrophication of the aquatic environment (Elmi *et al.,* 2015; Ebana *et al.,* 2017; Nehme et al., 2021). The soil can also be influenced by the discharge of heavy metals and other physicochemical assets resulting from machine processing. Normally, POME comprises nutrients such as sodium, phosphorus, and potassium that enhance plant growth, but high contents do not allow them to function properly, particularly at abnormal pH (Nwachukwu *et al.,* 2018). In spite of soil improvement by POME, the soil finds it difficult to support plant growth and becomes moist with organic remains leading to clogging and water logging of the soil pores and eventually resulting in death of the vegetation (Dahnoun and Djadouni, 2020). Such findings are reported in studies on POME by Ogunsina and Akintan, (2020) and Okitipupa, Nigeria and Elmi *et al.* (2015) from Malaysia. They point to the obvious fact that contamination of the environment with POME is worldwide.

Palm oil mill effluent is identified to possess high values of biological oxygen demand (BOD) and chemical oxygen demand (COD) (Ibrahim *et al.,* 2015). Biological oxidation of organic matter has led to the innovation of a low-cost secondary treatment of wastewater and industrial air emissions (Zainal *et al.,* 2017; Bhowmik *et al*., 2023). Studies have been documented on the use of POME at final release in composting by vermicomposting and combined composting with empty fruit bunch (EFB) (Adam *et al*., 2016; Nahrul *et al*., 2017). In the technologies involved in composting, the biomass, formed from palm oil, possesses a high nutrient value. It is important to invest on additional treatment systems for challenges like content of heavy metals in POME, selection of effective microorganisms, and management of leachate still persist even though the composting technology can be useful. This composting technology can generate more money for the trade, which can sometimes assist in reducing the biogenic component of POME (Nahrul *et al*., 2017).

Microorganisms are known to gain energy and carbon through the oxidation of organic compounds that are bioavailable in the environment. However, this process is dependent on a number of environmental and climatic factors (Ikhajiagbe et al., 2021). *Pseudomonas aeruginosa, Bacillus subtilis, Acinetobacter iwoffi, Flavobacterium* sp.*, Alcaligenes* sp.*, Aspergillus* sp., and *Rhizopus* sp. are effective in degrading environmental contaminants

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(Enerijiofi *et al.,* 2017; Enerijiofi, et al., 2022; Enerijiofi and Ikhajiagbe, 2021). The utilization of microorganisms in the natural treatment of POME in this current research offers a good alternative to decrease the oxygen demand and the natural burden content of the effluent as reported earlier (Ejeagba and Ihejirika, 2021). Ujiogba community is located in Esan land, Edo State of Nigeria. The community has been involved in the processing of oil palm for decades though at a subsistent level with the POME being emptied into the soil and underground water bodies. However, the reduction in biological and chemical oxygen demand capabilities of POME is very important as it improves the carbon-to-nitrogen ratio thereby utilizing it for improving soil fertilization and boosting agricultural production. This research aimed to utilize selected bacterial and fungal isolates to improve the oxygen demand capabilities of palm oil mill effluent.

## 2. Materials and Methods

### *2.1 Description of study area*

Ujiogba is one of the agrarian communities in Esan West Local Government Area of Edo State. It lies on latitude 6.5198°N and longitude 6.1315°E. Palm oil mill effluent (POME) samples were collected at three different processing sites at Ujiogba, Esan West local government area of Edo State, Nigeria. These palm oil mill sites have existed for more than ten years with POME from the mill being discharged regularly on the receiving soil.

### *2.2 Collection of samples*

Palm oil mill effluents were collected from three different palm oil processing mill sites at Ujiogba Edo state. Sterile plastic four-litre containers were used to collect the palm oil mill effluent sample in triplicate. POME contaminated soils were collected in polythene bags. Soil samples that were not polluted with POME served as control. The POME samples were instantly transferred to the laboratory for physiochemical and microbiological analyses.

### *2.3 Determination of total heterotrophic bacterial and fungal count*

Tenfold serial dilution of palm oil mill effluent (POME) contaminated soils were prepared, thereafter, 0.1 ml of $10^{-3}$, $10^{-6}$ and$10^{-9}$ diluents were plated into nutrient agar and potato dextrose agar plates respectively (Enerijiofi *et al*., 2022). The nutrient agar plates were supplemented with 50 µl of nystatin to inhibit fungal growth while the potato dextrose agar plates were supplemented with100 µl of streptomycin to inhibit bacterial growth. Bacterial isolation was conducted using nutrient agar plates, which were subsequently incubated at 37ºC for 24 hours. In contrast, fungal isolates were cultivated on potato dextrose agar plates at 28ºC for 72 hours. The discrete bacterial isolates were characterized and identified at the species level through cultural, morphological, and biochemical tests. The tests included shape, colour, margin, opacity, elevation, gram stain, arrangement, catalase, oxidase, indole, urease, citrate, lactose, sucrose, maltose, sorbitol, glucose, mannitol, and Voges Proskaeur (Holt *et al.,* 1994). In addition, the fungal isolates characterized and identified the species level through the microscopic and macroscopic methods like colour, shape, and arrangement of hyphae (Barnett and Hunter, 1972).

### *2.4 Characterization of POME*

This characterization was done before and after the treatment to determine the efficiency of the treatment with the bacterial and fungal isolates. The samples were collected and analyzed using standard methods in order to monitor the treatment process. The Biochemical Oxygen Demand (BOD) of the raw POME was determined using the HACH model 2173 BOD measurement apparatus while Chemical Oxygen Demand (COD) was determined by the titrimetric method as described in the standard method for the examination of water and wastewater (APHA, 2005; Ibegbulam-Njoku *et al.,* 2014b).

### *2.5 Bio-treatment of POME*

Each bacterial and fungal isolate of inoculum size 0.1ml ($10^6$ spores/ml) was introduced into 500 ml Erlenmeyer flasks containing 100 ml each of raw POME. They were incubated at 30ºC on a rotary shaker (200 rpm). The bio-treatment study was done for 15 days with samples drawn at 3-day intervals to determine the changes in biological oxygen demand (BOD) and chemical oxygen demand (COD) (Elmi *et al.,* 2015). The POME samples without the identified bacterial and fungal isolates served as controls for the bio-treatment studies.

### *2.6 Determination of bio-treatment efficiency*

The efficiency of organic load (BOD or COD) reduction was calculated following the method of Elmi *et al.* (2015).

Percentage efficiency = (X-Y)/X

Where X = initial (BOD or COD) of the raw POME; Y= final (BOD or COD) after treatment of POME

## 3. Results

The total heterotrophic bacterial count was 5.2 ± 0.12 cfu/g while the heterotrophic fungal count was 3.24 ± 0.20 cfu/g as documented in Table 1. The bacterial isolates identified were *Acetobacter* sp., *Pseudomonas* sp., *Micrococcus* sp., *Corynebacterium* sp. and *Bacillus* sp. (Table 2) while the fungal included *Aspergillus* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Penicillium* sp. (Table 3). *Pseudomonas* sp. was the most prevalent bacterial isolate (24.35%), followed by *Bacillus* sp. with 23.08% and the least in *Acetobacter* sp. (15.38%). *Penicillium* sp. was the most prevalent fungal isolate (33.60%), followed by *Aspergillus* sp. with 28.00% and the least in *Saccharomyces* sp. 17.60% (Table 4). The bio-treatment potentials of the bacterial and fungal isolates to treat palm oil mill effluent by measuring biological oxygen demand (BOD) are recorded in Table 5. *Bacillus* sp. and *Pseudomonas* sp. had percentage reduction values of 60.79% and 58.65% while a consortium of *Bacillus* sp. and *Pseudomonas* sp. had a percentage reduction value of 72.37%. *Aspergillus* sp. had a percentage reduction value of 65.75%, *Penicillium* sp. had 70.19% while the consortium of *Aspergillus* sp. and *Penicillium* sp. had a percentage reduction of 72.89%. The bio-treatment potentials of the bacterial and fungal isolates of palm oil mill effluent by measuring chemical oxygen demand (COD) are recorded in Table 6. *Bacillus* sp. and *Pseudomonas* sp. had percentage reduction values of 70.67% and 72.80% while a consortium of *Bacillus* sp. and *Pseudomonas* sp. had a percentage reduction value of 75.31%. *Aspergillus* sp. had a percentage reduction value of 50.26%, *Penicillium* sp. had 73.79% while the consortium of *Aspergillus* sp. and *Penicillium* sp. had a percentage reduction value of 63.05%.

**Table 1.** Enumeration of bacterial and fungal count from palm oil mill effluent contaminated soil (x$10^6$ cfu/g).

| Count | Mean ± standard error | Control |
|---|---|---|
| THBC | 5.20 ± 0.12 | 0.5 ± 0.01 |
| THFC | 3.24 ± 0.20 | 0.4 ± 0.11 |

**Legend:** THBC – Total Heterotrophic Bacterial Count; THFC – Total Heterotrophic Fungal Count.
Values represent mean ± standard error of the triplicate sample

**Table 2.** Cultural, Morphological, and Biochemical Characteristics of Bacterial Isolates.

| Characteristics | A | B | C | D | E |
|---|---|---|---|---|---|
| **Shape** | Circular | Circular | Circular | Irregular | Circular |
| **Colour** | Milky | Pale green | Milky | Pink | Milky |
| **Margin** | Entire | Entire | Lobate | Crenate | Lobate |
| **Opacity** | Opaque | Translucent | Opaque | Translucent | Opaque |
| **Elevation** | Flat | Flat | Flat | Flat | Flat |
| **Wet/dry** | Wet | Wet | Dry | Dry | Dry |
| **Gram stain** | + | - | + | + | + |
| **Shape** | Ova | Rod | Cocci | Rod | Rod |
| **Arrangement** | Chains | Pair | Single | Single | Pair |
| **Catalase** | - | - | + | + | + |
| **Oxidase** | + | + | + | + | + |
| **Indole** | - | + | + | + | + |
| **Urease** | + | - | - | + | - |
| **Citrate** | - | + | - | - | - |
| **Lactose** | - | + | + | + | + |
| **Sucrose** | + | + | - | - | + |
| **Maltose** | - | + | + | + | - |
| **Sorbitol** | + | - | - | - | + |
| **Glucose** | + | + | + | + | + |
| **Mannitol** | - | - | - | - | + |
| **Fluorescent** | - | + | - | - | - |
| **Amylase** | - | + | - | + | + |
| **Voges Proskaeur** | + | - | + | + | - |
| **$H_2S$ Production** | - | - | - | + | - |
| **Spore** | - | - | - | - | + |
| **Probable Identity** | ***Acetobacter* sp.** | ***Pseudomonas* sp.** | ***Micrococcus* sp.** | ***Corynebacterium* sp.** | ***Bacillus* sp.** |

**Table 3.** Microscopic and Macroscopic Characteristics of Fungal Isolates.

| S/N | Cultural Morphology | Microscopic examination | Probable Identity |
|---|---|---|---|
| 1 | Black fluffy colonies with reverse side yellow | Simple septate and branched conidia in chains. | ***Aspergillus sp.*** |
| 2 | White and cottony hyphae with reverse side white | Non septate hyphae with sporangiosphore and rhizoid | ***Rhizopus sp.*** |
| 3 | Milky whitish colonies, and white on reverse | Spherical to ova shape fungi<br>Stained positive on gram reaction | ***Saccharomyces sp.*** |
| 4 | Green flat colony with reverse side dirty white | Brush-like conidia, septate branching conidi-ophore was smooth/rough walled. | ***Penicillium sp.*** |

**Table 4.** Percentage Prevalence of Bacterial and Fungi Isolates from Palm Oil Mill Effluent Contaminated Soil (%).

| Bacterial species | Palm oil mill effluent n (%) | Control n (%) |
|---|---|---|
| *Acetobacter sp.* | 24 (15.38) | 2 (15.38) |
| *Pseudomonas aeruginosa* | 38 (24.35) | 4 (30.77) |
| *Micrococcus sp.* | 30 (19.23) | 3 (23.07) |
| *Bacillus* sp. | 36 (23.08) | 3 (23.07) |
| *Corynebacterium sp.* | 28 (17.95)<br>156 | 1 (7.69)<br>13 |
| **Fungal species**<br>*Aspergillus* sp. | 35 (28.00) | 4 (30.76) |
| *Rhizopus* sp. | 26 (20.80) | 3 (23.10) |
| *Saccharomyces* sp. | 22 (17.60) | 2 (15.38) |
| *Penicillium* sp. | 42 (33.60)<br>125 | 4 (30.76)<br>13 |

**Table 5.** Biological Oxygen demand (BOD5) values of Palm oil mill effluent with bacterial and fungal isolates over 15 days at 3- day intervals (mg/l).

| | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | (%) BOD reduction |
|---|---|---|---|---|---|---|---|
| **Bacterial isolates.** | | | | | | | |
| *Pseudomonas* sp. | 301.17±0.42 | 293.11±0.10 | 262.15±0.56 | 211.22±0.18 | 142.14±0.31 | 118.45±0.12 | 60.79 |
| *Bacillus* sp. | 339.33±0.89 | 283.24±0.31 | 251.05±0.59 | 215.28±0.47 | 162.10±0.52 | 140.31±0.67 | 58.65 |
| *Pseudomonas* and *Bacillus* | 398.28±0.02 | 311.12±0.44 | 242.70±0.18 | 194.13±0.76 | 152.05±0.38 | 110.04±0.41 | 26.92 |
| Control | 158.86±0.19 | 136.73±0.23 | 115.07±0.17 | 106.89±0.62 | 101.17±0.43 | 99.74±0.17 | 37.22 |
| **Fungal isolates.** | | | | | | | |
| *Aspergillus* sp. | 132.12±0.10 | 118.23±0.32 | 101.60±0.34 | 96.52±0.52 | 75.32±0.67 | 45.25±1.15 | 65.75 |
| *Penicillum* sp. | 128.23±0.54 | 114.31±0.25 | 98.74±0.62 | 65.19±0.21 | 55.32±0.49 | 38.22±2.01 | 70.19 |
| *Aspergillus* and *Penicillum* | 162.80±0.23 | 143.82±0.51 | 115.45±0.83 | 86.23±0.17 | 67.36±1.31 | 44.12±1.03 | 72.89 |
| Control | 105.76±0.19 | 88.11±1.11 | 71.26±0.16 | 49.37±0.42 | 32.37±2.13 | 60.73±0.45 | 42.58 |

**Legend:** Values represent mean ± standard error of triplicate samples

**Table 6.** Chemical oxygen demand (COD) values of palm oil mill effluent with bacterial and fungal isolates over 15 days at 3- day intervals (mg/l).

| | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | % COD reduction |
|---|---|---|---|---|---|---|---|
| **Bacterial isolates.** | | | | | | | |
| *Pseudomonas* sp. | 206.12±0.41 | 187.23±0.32 | 146.11±0.89 | 123.99±1.23 | 96.06±1.22 | 56.06±2.10 | 72.80 |
| *Bacillus* sp. | 198.21±1.11 | 164.30±0.54 | 120.05±0.22 | 98.55±0.45 | 77.58±0.34 | 58.14±0.39 | 70.67 |
| *Pseudomonas* and *Bacillus* | 245.51±0.45 | 202.91±0.58 | 185.80±0.59 | 137.74.±0.67 | 102.62±0.81 | 60.62±0.81 | 75.31 |
| Control | 110.12 ±0.66 | 96.12 ±0.54 | 78.06±0.33 | 66.53±0.86 | 54.13±0.94 | 70.29±0.94 | 36.17 |
| **Fungal isolates.** | | | | | | | |
| *Aspergillus* sp. | 191.97±0..85 | 182.53±2.11 | 162.63±1.25 | 125.48±0.56 | 111.48±0.77 | 95.48±0.32 | 50.26 |
| *Penicillium* sp. | 198.01±0.30 | 178.91±3.01 | 159.33 ±0.52 | 123. 66±0.46 | 99.67±0.15 | 51.88±0.15 | 73.79 |
| *Aspergillus* and *Penicillium* | 232.48±49 | 206.89±.58 | 185.82±1.83 | 144.67±2.08 | 111.59. ±1.70 | 85.91. ±2.70 | 63.05 |
| Control | 121.66±2.12 | 105.64±1.11 | 92.33±2.10 | 73.86±0.16 | 56.94±1.20 | 80.94±0.32 | 33.47 |

**Legend:** Values represent mean ± standard error of triplicate samples

## 4. Discussion

The total heterotrophic count revealed that the palm oil mill effluent (POME) contaminated soils had more heterotrophic bacterial and fungal loads than the control. This output indicated that POME contained more nutrients for bacterial and fungal growth and multiplication hence higher counts more than the control. The results of this study concurred with those of earlier authors, Orji *et al.* (2006), Ibegbulam-Njoku, and Achi, (2014b) who reported that palm oil mill effluent enhanced microbial growth. Palm oil mill effluent (POME) is of great interest to many researchers because of high levels of biogenic matter, which indicates high BOD and COD values as well as oil and grease, which serve as substrates for microorganisms. Oil in palm oil mill effluent is an excellent source of carbon with about twice the energy value of glucose during microbial growth (Ibegbulam-Njoku and Achi, 2014b). The organic nature of palm oil mill effluent (POME) indicates that the effluent is biologically degradable, and laboratory experimental findings have confirmed it (Orji *et al*., 2006). The findings also showed that soils with older POME deposits tend to have more numbers and varied microorganisms than nearby soils without POME deposits. This difference is because palm oil mill effluents are rich in biodegradable organic matter and as the micro-organisms biodegrade the organic content of the POME, the receiving soil is inevitably influenced.

This study documented different bacteria and fungi isolates from palm mill effluent-contaminated soil. These include *Pseudomonas, Acetobacter, Micrococcus, Bacillus, Corynebacterium, Aspergillus, Rhizopus, Saccharomyces* and *Penicillium* species. The percentage of prevalence had *Pseudomonas* sp. as the highest (24.35%), followed by *Bacillus* sp. with 23.08% and least in *Acetobacter* sp. (15.38%). *Penicillium* sp. was reported to as the most prevalent fungal isolate (33.60%), followed by *Aspergillus* sp. with 28.00% and least in *Saccharomyces* sp. with 17.60%. The different bacterial and fungi isolate could be because the palm oil mill effluent is disposed to degradation by microorganisms resulting from its organic nature as reported earlier (Loretta *et al*., 2016). The variation in the microorganisms isolated in this study and those of earlier researchers could be attributed to the nature of the environment, whether the microorganisms were mesophilic or thermophilic, and the population changes along the disposal channel. This is in line with the observations of Ewelike *et al.* (2021) who noted that the nature and behavior of the microbial population in the POME environment are influenced by many physiochemical parameters of ecological importance.

The bio-treatment abilities of the selected bacterial and fungal isolates to treat palm oil mill effluent were measured using two parameters: biological oxygen demand (BOD) and chemical oxygen demand (COD). Biological oxygen demand and Chemical oxygen demand indicate organic pollutants in wastewater. Bacteria utilize the organic compounds in the wastewater for growth and metabolism. In the BOD, *Bacillus* sp. and *Pseudomonas* sp. had percentage reduction values of 60.79% and 58.65% respectively while the consortium of *Bacillus* and *Pseudomonas* species gave 72.37% reduction. *Aspergillus* sp. had a percentage reduction value of 65.75%. *Penicillium* sp. gave 70.19% while the consortium of *Aspergillus* and *Penicillum* species had a percentage reduction of 72.89%. However, for the COD, *Bacillus* sp. and *Pseudomonas* sp. had percentage reductions of 70.67% and 72.80% respectively while their consortium gave a percentage reduction of 75.31%. *Aspergillus* sp. had a percentage reduction value of 50.26%, *Penicillium* sp. had 73.79% while the consortium of *Aspergillus* and *Penicillium* species had a percentage reduction of 63.05%. This study revealed that the consortium of the microbial isolates; bacterial-bacterial and fungal-fungal had a higher percentage reduction in both monitored parameters; biological oxygen demand and chemical oxygen demand than the individual isolates. The findings in this study concurred with the earlier submission of Karim *et al*. (2019) who reported that *Bacillus cereus* through the batch fermentation system substantially reduced contamination burden precisely COD and BOD for 50% (v/v) palm oil mill effluent within 6 days.

However, since POME is said to be non-toxic as it does not involve the addition of chemicals during the oil extraction process, it uses up the bioavailable oxygen in the aquatic environment for the degradation process, thereby providing a noble nutrients source for microbial growth and subsequently leading to a reduction in the COD and BOD. It also increases the organic nitrogen available, which supports plant growth and development (Razak *et al*., 2022; Elmi *et al.,* 2015). Therefore, the results revealed that treatment of POME before discharge would reduce the risk of pollution as corroborated by the submission of Karim *et al.* (2019) that bio-treatment of palm oil sludge using selected microorganisms on continuous fermentation has been shown to improve the quality of the effluent.

## 5. Conclusion and Recommendation

The study revealed that the POME-contaminated soils contained *Pseudomonas, Acetobacter*, *Micrococcus, Bacillus*, *Corynebacterium*, *Aspergillus*, *Rhizopus*, *Saccharomyces,* and *Penicillium* species. It also reports treatment of the final discharge palm oil mill effluent focusing on COD and BOD reduction using *Pseudomonas, Bacillus*, *Aspergillus,* and *Penicillium* species as revealed by the substantial reduction in their concentrations. Based on these findings, bio-treatment of POME is recommended to prevent environmental contamination and to improve the carbon-to-nitrogen ratio of the effluent making it good for improving soil fertility.

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