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Biophysical Characterization of Palm Oil Mill Effluent from Adapalm, Imo State Nigeria

Keywords: Environment; Fungi; Physicochemical; Waste discharge

Abstract

This study was designed to investigate the biophysical characteristics of Palm Oil Mill Effluent (POME) produced by ADAPALM oil mill, Imo State, Nigeria. A total of three samples were ascetically collected from the mills. The POME was subjected to standard microbiological analysis and physiochemical studies. The population of Total Heterotrophic Bacteria (THB) ranged from 1.71 x 104 - 1.90 x 104 (cfu/g), the Total Heterotrophic Fungi (THF) ranged from 4.70 x 102 - 7.00 x 102 (cfu/g). The bacteria isolated from the POME followed the order *Staphylococcus* sp. (38%) >*Pseudomonas* sp. (32%) > *Serratia* sp. and *Bacillus* sp. (15%), while the fungi isolate from POME include *Aspergillus niger, Aspergillu flavu, Penicillium Specie* and *Mucor species*. The physiochemical characteristics showed range of 3.98 ± 0.03 to 4.22 ± 0.02 for pH, electrical conductivity from 291 ± 0.8 to 312 ± 1.6 µS/cm, temperature from 27.2 ± 28.8 ± 0.1 oC and moisture content from 4.1 ± 0.4 to 6.0 ± 0.1% respectively. Although, the POME contains microbial species capable of degrading hydrocarbons in the POME to prevent environmental impacts, the results showed possible non existence of effluent treatment of any form.

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Ihenetu SC, Enyoh CE*, Uzoigwe AD, Ubah OG

[1]*Department of Chemistry, Imo State University Owerri, Nigeria*
[2]*Department of Agronomy, Micheal Opara University of Agriculture Umudike, Nigeria*
[3]*Department of Microbiology, University of Portharcourt, Nigeria*

***Address for Correspondence**
Enyoh Christian Ebere, Group Research in Analytical Chemistry, Environment and Climate Change (GRACE&CC), Department of Chemistry, Imo State University Owerri, Nigeria; E-mail: cenyoh@gmail.com

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Introduction

The palm oil (*Elaeis Guineensis*) industry in Nigeria is very important and vital for the Nigerian economy, which represents 3% of the world production in 2010 [1]. Thus increasing its cultivation in many parts of Nigeria especially the Niger Delta. With the economic benefits comes environmental problem associated with effluents from the industry. These effluent products consist of solids materials such as Empty Fruit Bunches (EFB), Palm Pressed Fibers' (PPF), Palm Kernel Shell (PKS), Palm Kernel Cake (PKC) and liquid effluents such as Palm Oil Mill Effluent (POME) [1-5].

Palm oil mill effluent is the voluminous liquid waste that comes from the sterilization and clarification processes in milling oil palm. The raw effluent contains 90-95% water and includes residual oil, soil particles and suspended solids [6]. About 2.5 t of effluent per tonne of palm oil, or 0.5 tonne of effluent per tonne of fresh fruit is produced by a palm industry. POME is a highly polluting material and it continuous deposition in an area will cause the area to be abandoned and fresh space is located. Several reports have stressed the ecological and pollution hazards associated with this disposal method [7].Thus prompting much research dedicated to the means of alleviating its threat to the environment through assessing its physicochemical and biological properties. These are essential characteristics to be investigated before discharging POME to the recipient environment [8].

Several studies have been conducted on POME from different palm extraction sites [7-11]. While studies in I'm state has been limited. Verla and coworkers studied POME randomly collected at source and from a dump site in Nguru, AbohMbaise, Imo state Nigeria for physicochemical and microbial properties [4]. Results revealed that POME characteristics were far above Environmental Health and Safety guidelines, which indicates unhealthy environment conditions with potential negative consequences for humans and the ecosystem. There is therefore need for environmental agencies and governments to take appropriate preventive measures to avert potential problems due to indiscriminate dumping of POME [12]. The aim of this study is to characterize POME from Adapalm. As far as it could be established no study has reported on the characteristics of POME from Adaplam. Therefore, this paper reports the physicochemical, biochemical and microbial qualities of POME from Adapalm, Imo state Nigeria.

Materials and Methods

Site description and sample collection

The study area is located at Ohaji Egbema Local Government Area of Imo State. The farm was established in 1962 as Ohaji Farm settlement and a size of 4319 hectares of plantation [13]. Geographically, the area lies within coordinates of 5.3138 °N, 6.8780 °E.

Three (3) samples of Palm Oil Mill Effluent generated were collected ascetically with sterile 500 ml bottles from different mills in adapalm which were labeled A, B and C and were taken to the Imo state university microbiology laboratory where they were analyzed.

Quality control

All chemicals used for this work were analytical grade chemicals purchased from Fin. Lab and ChemiScience, Owerri and were used without further purification. All glassware and media used were sterilized by autoclaving at 121°C for 15 mins at 15 pounds pressure and air drying in the hot air oven at 160 °C for 2 hrs.

Methods for physical analysis of POME

Determination of POME temperature: The thermometer was inserted inside the sample and it was allowed to stay for about 5 minutes before taking the temperature reading of the sample.

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Table 1: Total bacterial and fungal counts for all the POME samples.

POME Samples	Bacteria count (cfu/g)	Fungi count (cfu/g)
Sample A	1.90 x 10^4	7.00 × 10^2
Sample B	1.71 x 10^4	6.20 x 10^2
Sample C	1.86 x 10^4	4.70 × 10^2

Table 2: Bacterial isolates.

S/N	Microorganism isolated	No of isolate	% isolated
Sample A			
1	*Staphylococcus spp.*	16	37.2
2	*Pseudomonas spp.*	14	32.5
3	*Seratia spp.*	5	11.6
4	*Bacillus spp.*	8	18.6
Sample B			
1	*Staphylococcus spp.*	13	37.1
2	*Pseudomonas spp.*	11	31.4
3	*Seratiaspp.*	6	17.1
4	*Bacillus spp.*	5	14.3
Sample C			
1	*Staphylococcus spp.*	14	38.9
2	*Pseudomonas spp.*	12	33.3
3	*Seratiaspp*	6	16.6
4	*Bacillus spp.*	4	11.1
Total Isolate			
1	*Staphylococcus spp.*	43	38
2	*Pseudomonas spp.*	37	32
3	*Seratiaspp.*	17	15
4	*Bacillus spp.*	17	15

Determination of electrical conductivity: The electrical conductivity was determined by using the HANNA HI8733 electrical conductivity. The electrical conductivity meter was first calibrated using solution of potassium chloride [14]. 200 ml of the sample was measured into 500 ml beaker and it was allowed to stay for about 30 minutes. The electrode was dipped into the beaker and the reading was obtained from the electrical conductivity screen.

Determination of the pH: The pH meter was first standardized in buffer solution of 4,10 and 7. 150 ml of the sample was weighed into 250 ml beaker and was stirred for about 30 minutes and it was allowed to stay for 2 hours. The pH electrode was dipped into the beaker and it was allowed to stand for about 5 minutes before taking the reading [14-15].

Moisture content: Aliquot of 10 ml of each sample was added into a silica dish. This was immersed in a water bath until the water was completely evaporated. The dried solids in the silica dish was then removed from the water bath and dried at 70 °C in a vacuum oven for 3 hours and then placed in the desiccators for 10 minutes, for cooling. The content of the dish was weighed and the moisture content calculated as follows [16].

$$\% \text{ moisture content} = \frac{\text{weight of sample after drying}}{\text{weight of sample before drying}} \text{ x } \frac{100}{1}$$

Microbiological analysis of samples

Total heterotrophic bacteria count and fungi count: The populations of microorganisms in the samples were enumerated using serial dilution pour plate method. About 0.1 ml of POME sample was serially diluted in sterile distilled/deionized water and aliquots of the dilutions were ascetically plated into the 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 and 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. Microbial colonies were isolated into pure cultures and preserved in slants for further analysis [16].

Identification of isolates

The samples were identified using their morphological and cultural identification and further subjected to biochemical characterization.

Data analysis

Analysis of Variance (ANOVA) was used to test significant differences at 5% level of significance.

Result and Discussion

Microbiological result

The microbial population of POME is presented in (Table 1). The total bacteria count ranges from 1.71 x 10^4 - 1.90 x 10^4 (cfu/g). The total fungi count ranges from 4.70 x 10^2 - 7.00 x 10^2 (cfu/g).

From the result obtained from the total bacteria count ranges from 1.71 x 10^4 - 1.90 x 10^4 (cfu/g) (Table 1). The total fungi count ranges from 4.70 x 10^2 - 7.00 x 10^2 (cfu/g). The result showed that all the samples collected from various mills have similar microbial species

Identification of bacterial isolates

The result in showed that all the samples collected from various mills have similar microbial species (Table 2). The bacteria isolated from the POME include *Pseudomonas* sp.,*Serratia* sp, *Bacillus* sp, and *Staphylococcus* sp.

The result obtained from shows that all the samples collected from various mills have similar microbial species (Table 2). The bacteria isolated from the POME followed the order *Staphylococcus* sp. (38%)

Table 3: Biochemical test of bacteria isolates of POME.

Organism	Gram reaction	Mor	Oxi	Cat	Cit	Coa	Ure	Ind	Glu	Gal	Lac
Pseudomonas sp	Negative rod	+	+	+	+	-	-	-	A	-	-
Serratia sp	Negative rod	+	+	+	+	-	-	-	A/G	A/G	A
Bacillus sp	Positive rod	+	+	+	+	-	-	-	A	A/G	A
Staphylococcus sp	Positive cocci	-	-	+	-	+	-	-	A/G	A/G	A/G

Keys: +: Positive; -: Negative Reaction; A: Acid; A/G: Acid and Gas; Oxi: Oxidase; Cat: Catalase; Cit: Citrate; Coa: Coagulase; Ure: Urease; Ind: Indole; Glu: Glucose; Gal: Galactose; Lac: Lactose; Mo: Motility

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Table 4: Microscopic morphology and cultural characteristics.

Organisms	Microscopic morphology	Cultural characteristics
Aspergillus	Presence of septate hyphae; long and smooth conidiophores, long unbranded sporangiospores with large, round head.	Creamy to brownish-black mycelium with dark spores and often appears golden on the reverse side.
Niger		
Aspergillus	Presence of septate hyphae, colourless and rough conidiophores with swollen vesicles.	A greenish-yellow colour with a creamy edge. That appears golden in the reverse of the septate
Flavus		
Mucorspecies	Presence of non septate hyphae with a visible spore and short	A creamy colonies that covers the entire medium and they are irregular in shape.
	sporangiosphores.	
Penicillium	Presence of septate and fruity mycelium and branched conidiophores. It has a red pigment, and the edge is surrounded by	A greenish filament is seen which changes to powdery
Species	whitish margin	Greenish brown after days and it is yellow on the reverse side.

>*Pseudomonas* sp. (32%) > *Serratia* sp/*Bacillus* sp (15%) There was abundant occurrence of *Staphylococcus* sp in all samples compared to all other bacterial isolated. Our results showed variation to results of and [4,19]. Eze (19) reported highest occurrence of *Pseudomonas aeruginosa* (27%) followed by *Staphylococcus aureus* / *Klebsiella* species (17%), while Proteus species [19], *Bacillus* species, *Citrobacter* species have the lowest percentage occurrence of 6% in POME from Umuahia, Abia State, Nigeria. Sinnapa showed that the variation of microorganism in POME sites could be attributed to the nature of the environment and that the population changes along disposal channel [18]. Another reason is the acclimatization and adaptation of these microorganisms' older POME deposits as suggested by Orji and Ihenetu [9,16].

Biochemical test results

The biochemical test carried out for this microbiological analysis *Pseudomonas spp, Seratia spp, Staphylococcus spp*, and *Bacillus spp*. include catalase test, indole test, coagulase test, methyl red test, citrate test, motility test, urease test and sugar fermentation test. The biochemical identification of microorganisms gives us an idea of what these microorganisms are able to do, being possible the discrimination of different strains of the same species by specific biochemical profiles. Differences in concrete enzymatic activities tell us about the ecology, the physiology or the natural habitat of the microorganism.

(Table 3) shows the biochemical test carried out include gram staining, motility test, oxidase, catalase, citrate, coagulase, urease, indoleteste and sugar fermentation test. The fungal isolates include *Aspergillus niger*, *Aspergillus flavus, Mucor* species and *Penicillium* species. The fungal isolate was similar to fungal isolated in POME from Nguru reported by Verla [4].

Microscopic morphology and cultural characteristics of fungi isolates.

The result below shows the microscopic and cultural characteristics of the fungi isolates (Table 4).

The physiochemical characteristics

The result below shows the physiochemical characteristics of the samples of palm oil mill effluent analyzed which were labeled sample A, B and C, which include the pH, electrical conductivity, temperature and moisture content.

From the result obtained from (Table 5), which shows the physiochemical characteristics which were calculated in line with their standard deviation for the samples obtained from the different palm oil mill effluents which were labeled sample A, B and C shows that the pH of the samples collected from different mill ranges from 3.98 ± 0.03 to

4.22 ± 0.02. The electrical conductivity of the samples ranges from 291 ± 0.8 to 312 ± 1.6. The temperature of the samples ranges from 27.2 ± 28.8 ± 0.1. Lastly, the moisture content of the samples ranges from 4.1 ± 0.4 to 6.0 ± 0.1. Same superscripts for values in the same column are not significantly different ($P<0.05$).

The results obtained for pH showed that the POME was strongly acidic. This observation is in contrast to results of [4], were a pH range of 6.8 ± 0.22 to 8.7 ± 0.28 showing weakly acidic to weakly alkaline reported for POME in Nguru, Aboh Mbaise, Eastern Nigeria. The receiving environment of high acidic POME will be adversely affected and thus may affect aquatic life or crops yield. pH is an important parameter that controls many chemical processes in environment matrix and therefore advisable that it undergoes some form of treatment or decomposition before discharge. When properly treated and packaged, it can be used by farmers both in rural and urban areas to improve soil fertility thereby increasing the agricultural productivity for global, national and regional food demands [19]. The treatment helps in avoiding the initial harsh effects of POME on soil meant for agriculture. There were no significant differences ($P<0.05$) between the different samples for pH. The electrical conductivity measures the dissolved material in the POME, which relates to the ability of POME to conduct electrical current through it. The EC recorded in the present study are higher to values reported by [4]. High EC indicates more dissolved materials in the studied samples. The dissolved materials include minerals inform of cations and anions and thus suggesting that POME could be useful as fertilizer at certain concentrations. Authors suggested that POME should be assessed carefully for potential mineral toxicity and thus it is important to always assess minerals in POME for toxicity before use [12,21].The EC follows the trend; C > B > A with only A found to be significantly different ($P >0.05$).

Conclusion

In this study, the population of microorganisms found in POME was enumerated. The POME had a high population of heterotrophic bacteria and fungi. The microbial isolates from study are the similar in all the processing mills with their occurrence following this order; *Staphylococcus* sp. (38%) >*Pseudomonas* sp. (32%) >*Serratia* sp and *Bacillus* sp (15%). These microbes have direct applications in industrial process such as bioremediation and biodegradation of oily wastewater. The physiochemical characteristics of samples obtained from different mills labeled A,B and C shows similar characteristics, which confirm the non existence of effluent treatment of any form.

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Table 5: Physiochemical characteristics of the sample.

POME samples	pH	EC µS/cm	Temp °C	Moisture
Sample A	4.01 ± 0.04[a]	291 ± 0.8[a]	28.8 ± 0.1[a]	5.2 ± 0.2[a]
Sample B	4.22 ± 0.02[a]	302± 1.1[b]	27.2 ± 0.3[b]	4.1 ± 0.4[b]
Sample C	3.98 ± 0.03[a]	312± 1.6[b]	28.4 ± 0.2[a]	6.0 ± 0.1[c]

Key: EC: Electrical Conductivity; Temp: Temperature
Same superscripts for values in the same column are not significantly different (P<0.05).

Some of the major obstacles to adoption of cleaner solutions in POME management in palm oil mills have been the total absence of sustainable technology and compelling economic arguments. There are reasons why POME has been treated and handled by millers as waste instead of resource POME has potential for use as medium for microalgae cultivation with significant saving in treatment costs. Due to variation in the composition of POME, the concentration that promotes the best biomass or product yield has to be pre-determined in order to reduce the negative impact of the inhibitory components on the metabolism of cultures. Therefore, POME should be considered a valuable resource, and recovering it for other uses is much more preferable environmental alternative than the current treatment and disposal.

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