EQA - International Journal of Environmental Quality
ISSN 2281-4485 - Vol. 44 (2021): 9-17
Journal homepage: https://eqa.unibo.it/

published by

Microbial load and biodegradation of palm oil mill effluent (POME) by microorganisms at different stages of discharge

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Article info
Received 24/10/2020; received in revised form 19/2/2021; accepted 16/3/2021.
DOI: 10.6092/issn.2281-4485/11667

Abstract
The raw palm oil mill (POME) effluent has an extremely high content of degradable organic matters as a result of unrecovered palm oil during production of palm oil. The aim of this study is to evaluate the microbial load and biodegradation of POME by microorganisms at different stages of discharge. Standard dilution methods were used for the isolation of bacteria and fungi. The estimation of colony forming unit per mL (cfu/mL) was assessed using 10-fold serial dilution method. The results showed that the total microbial count (TMC) decreased with time. Statistical analysis using analysis of variance (ANOVA) further revealed a significant variation in microbial load after 28 days of discharge. This was followed by a significant decrease after 36 days when compared with other samples. The physicochemical studies further revealed the presence of relatively low levels of sodium, potassium, calcium, magnesium and iron. The concentration of nitrate was relatively high, 70.13 mg/L. The biological oxygen demand (BOD) and the chemical oxygen demand (COD) were also high 2250mg/L and 3360mg/L respectively. High BOD and COD values are important indicators of high organic and inorganic components of the effluent and possible negative environmental consequences. The presence of the following species *Clostridium*, *Proteus*, *Pseudomonas*, and *Micrococcus* implied possible degradation of the effluent.

Keywords
palm oil mill, effluent, pollution, microbial load, biodegradation

Introduction

Oil palm production has been recognized for its contribution towards the economic growth and sustenance of most palm oil producing communities in Nigeria. Contrary to its economic benefits, it has also contributed to environmental pollution due to the production of huge quantities of by- products from the extraction process. The waste products from oil palm processing consist of oil palm trunk (OPT), oil palm front (OPF), empty fruit bunches (EFB), palm pressed fibers (PPF), palm kernel shell and less fibrous materials such as palm kernel cake (PKC) and liquid discharge palm oil mill effluent (POME). The constituents of raw POME have been reported to be a colloidal suspension of 95 -96% water, 0.6 - 0.7% oil and 4 - 5% total solids including 2 - 4% suspended solids (Ahmad et al. 2003). POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil, thus, POME should be treated before discharge to avoid serious environmental pollution (Ojonama and Nnennaya, 2007). According to Ahmed et al. (2003) the highly polluting wastewater can cause pollution of waterways due to oxygen depletion and other related effects.

In Nigeria, most production of palm oil is done

DOI: 10.6092/issn.2281-4485/11667

by individual families scattered throughout palm oil producing communities. Very few people can afford the mechanized hydraulic press system of oil extraction. Most processing methods are local and traditional, involving rudimentary equipment and the division of labour and tasks closely integrated with the domestic routine of a basically agricultural economy. POME produced by the small-scale traditional operators undergoes little or no treatment and is usually discharged into the surrounding environment (Ojonama and Nnennaya, 2007). The indiscriminate discharge of the effluent with its emanating offensive odor constitutes health hazard to the people and the environment. When discharged untreated, POME can alter the environmental parameters causing a change in water and soil qualities especially in the BOD, DO, C/N ratio and COD. These parameters according to Arias *et al.,* (2005), affects microbial flora, which in turn affects soil health.

A variety of microorganisms have been found to be capable of biodegrading oil waste water with high profits. However in the case of POME, there is need to investigate the various microorganisms responsable for the degradation at different stages as this will help in optimizing the biodegradation processes. This work is therefore aimed at determining the microbial load and biodegradation of POME at different stages of discharge and the impact on soil quality.

Materials and Methods

Sampling Collection

Palm oil mill effluent (POME) was obtained from an established oil mill on the outskirts of Owerri, Imo State, Nigeria. The effluent is normally discharge without treatment into a dumpsite. Samples were collected on the first day of discharge and subsequently 7, 14, 28, and 36 days after discharge into the dumpsite. Samples were collected in sterile dry, glass bottles. Collected samples were immediately labeled and transported to the laboratory for microbiological analysis.

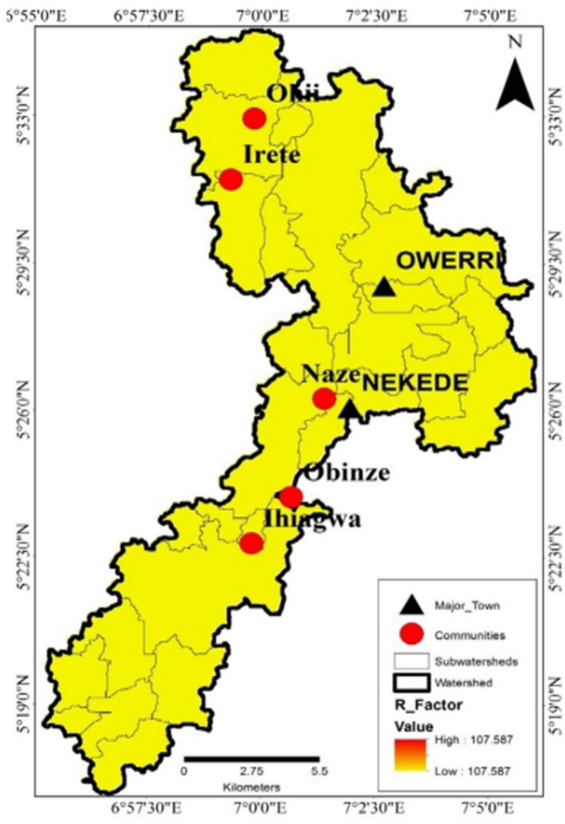


Figure 1. *Study area.*

DOI: 10.6092/issn.2281-4485/11667

Enumeration of Total Heterotrophic Bacteria and Fungi

The standard dilution plate method was used for isolation of bacteria in nutrient agar (NA) (Kamil *et al.* 2007) and fungi in potato dextrose agar (PDA) supplemented with lactic acid to discourage bacterial growth at different stages of degradation. 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. Observations and counting for bacteria colonies were carried out at 24 to 48h after inoculation. Counting of fungi colonies were carried out at 72h after inoculation and the plates were observed for seven days after inoculation for fungi identification before they were discarded. Viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming units per milliliter (cfu/mL) of the sample. District colonies were sub-cultured for isolation and purification.

Identification of bacterial and fungal isolates

The bacteria isolates were identified using biochemical test. The derived characteristics were compared with those known using manual of determinative bacteriology. Fungi identification was based on the macroscopic and microscopic morphology.

Determination of Chemical Characteristics of POME

The temperature, pH, conductivity, and turbidity were determined using digital meters. The TDS and TSS measurements were carried out by gravimetric method.

$$\text{TDS/L} = \frac{(\text{A-B}) \times 1000}{\text{mL Sample}} \qquad [1]$$

where: A= weight of dried residue + dish, mg, B= weight of dish, mg.

$$\text{TSS/L} = \frac{(\text{A-B}) \times 1000}{\text{mL Sample}} \qquad [2]$$

where: A= weight of filter + dried residue, mg, B = weight of filter, mg

Nitrate, phosphate, and sulphate were determined by using a spectrophotometer with Nitra var 5 nitrate, Phosphor var 5 phosphate, and Sulfa var 4 sulphate reagents as described in the HACH Water Analysis Handbook (APHA 2005). BOD_5 was determined using methods outlined by APHA method 5210. This was based on the oxidation reaction of organic substances with oxygen in the water in the presence of aerobic bacteria. COD is the amount of oxygen required to oxidize soluble and particulate organic matter in water. The value of COD is an indicator for water pollution by organic substances which can naturally be oxidized through microbiological processes and results in reduced oxygen dissolved in water. COD was determined by using the procedures outlined in APHA 2005.

Oil and Grease were determined as described by the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The amount of oil and grease can be calculated by the following formula:

$$\text{Oil and Grease} = \frac{(\text{A-B}) \times 1000}{\text{Sample test volume}} \qquad [3]$$

where: A is weight of empty Whatman paper, B is final weight of sample C is weight of initial sample.

Results

The result of the physicochemical parameters of POME is shown in Table 1. The bacteria isolated from the POME samples at different stages of decomposition are shown in Table 2. The bacteria isolated include *Bacillus subtilis*, *Clostridium* sp, *Streptococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Flavobacterium* sp., *Cellulomonas* sp, and *Micrococcus luteus*.

The aerobic count of samples ranged from 7.1×10^6cfu/ml – 1.9×10^6cfu/ml with samples collected 28days after discharge (D) having the highest bacteria load while samples collected a day after discharge (A) showed the least bacteria load. This is shown in Table 3. Figure 1 illustrates the TMC at different stages of decomposition. Table 4 shows the fungi isolated and identified. These include: *Aspergillus flavus, Rhizopus stolonifer, Epicoceum niger, Aspergillus niger, Aspergillus terreus, Aspergillus flumigatus, Mucor* sp, Yeast Cells, *Candida* sp, *Cladosporum* sp and *Histoplasma capsulatum.*

DOI: 10.6092/issn.2281-4485/11667

Table 1. *The physicochemical parameters of samples of POME with Federal Environmental Protection Authority (FEPA) standard.*

Parameter Guideline	Value*	FETA Effluent limitation
Temperature	33±0.01	> 40
pH	5.3±0.01	6 - 9
Oil & Grease	320±0.05	10
TSS (mg/L)	83±0.02	30
TSD (mg/L)	50±0.01	200
COD (mg/L)	3305±0.03	-
BOD (mg/L)	2275±0.02	50
NO_{3-} (mg/L)	70±0.03	20
SO4 (mg/L)	35±0.02	1000
Na (mg/L)	30±0.03	-
Ca (mg/L)	14±0.04	-
Mg (mg/L)	10±0.02	-
Fe (mg/L)	14±0.03	-

*Values are means of replicate determinationsSD

Table 2. *The bacterial isolates from POME samples at different stages of decomposition.*

Bacteria	Days				
	A1	B7	C14	D28	E36
Bacillus subtilis	+	+	-	+	+
Clostridium sp	-	-	-	-	+
Streptococcus aureus	+	-	-	+	+
Proteus vulgaris	+	+	-	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Flavobacterium sp	+	-	-	-	+
Cellulomonas sp	-	+	+	+	+
Micrococcus luteus	+	-	+	+	+

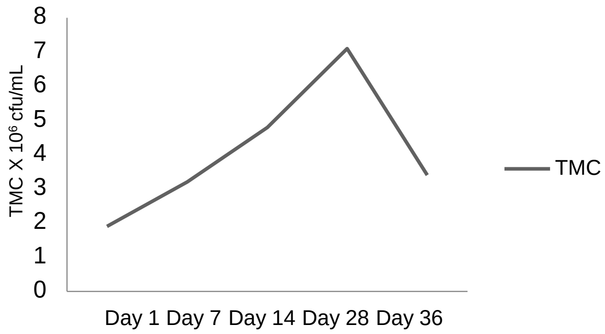
Legend: + = Present: - = Absent

Table 3. *Mean aerobic plate count of POME at different stages of decomposition.*

Results/ Days					
Total Microbial Count	A1	B7	C14	D28	E36
TMC x10^6 cfu/mL	1.9	3.2	4.8	7.1	3.4

Legends: Day 1=A, Day 7=B, Day14=C, Day28=D and Day36=E

DOI: 10.6092/issn.2281-4485/11667



Legends: Day 1=A, Day 7=B, Day14=C, Day28=D and Day36=E

Figure 1. *Total microbial count TMC at different stages of decomposition.*

Table 4. *The fungal isolates from POME samples at different stages of decomposition.*

Fungal isolates	Days				
	A1	B7	C14	D28	E36
Rhizopus stolonifer	+	-	-	+	+
Phoma glomerate	+	+	-	-	+
Epicoceum nigrum	-	+	-	-	-
Aspergilus terreus	-	+	+	+	-
Aspergillus niger	+	+	+	+	+
Mucor racemosus	+	+	+	+	+
Yeast	+	+	+	+	+
Brettanomyces intemedium	-	-	-	-	+
Candida albicans	+	+	-	+	+
Mucor racemosus	+	+	+	+	+
Cladosporum species	-	+	-	+	+
Histoplasma capulatum	-	-	-	+	+

Legend: + = Present: - = Absent

Discussion

Treatment and disposal of oily wastewater, such as palm oil mill effluent is presently one of the serious environmental problem facing many villages where small scale palm oil production is their main stake. Disposal of untreated palm oil mill wastes have existed for years in these villages, but their effects on environment are at present more noticeable due to activities of environmental regulatory authorities.

The parameters determined as shown in Table 1 are among the parameters listed in the guideline for effluents limitation by Federal Environmental Protection Agency, Nigeria (FEPA). The mean pH value of the effluent sample was 5.3 at the point of discharge. This is slightly acidic and might suggest why raw POME suppresses the growth of crops when freshly applied in the environment.

The TSS (83±0.02) of the POME was above the FEPA standard (30 mg/L). TDS (50±0.01) was below the FEPA standard. The high COD level of the POME (3305±0.03mg/L) suggests the high concentration of chemically oxidable constituents in the POME. The strength of wastewater is judged by its BOD and this defined as oxygen required by bacteria while stabilizing the organics in the wastewater under aerobic conditions at a particular temperature and time (Verla *et al.* 2018). Therefore, the high BOD level observed is suggestive

DOI: 10.6092/issn.2281-4485/11667

and indicative of high level of biodegradable compounds in the POME. The presence of the metals K, Na, Ca, Mg and Fe at restively moderate concentration is in corroboration with earlier reports (Jeremiah *et al.* 2018). A variety of microorganisms such as bacteria, molds, and yeasts, have been shown to be capable of completely degrading oil wastewater (Jeremiah *et al.* 2018). The high organic matter in POME wastewater possibly will have played an essential role in the abundance of aerobic and facultative anaerobic microbial strains in the present study.

The microorganisms isolated from POME at different stages of decomposition, has the ability to degrade carbon source present in the POME. Bala (2016) reported that *B.cereus* and *B. subtilis* has the ability to degrade and utilize lipid as carbon source. *B. subtilis* was isolated from sample A, B, D and E. The isolation of this lipase producing bacteria from all the samples suggests that the oily nature of the POME may have served as a good medium for lipolitic microorganisms to thrive (Mukesh *et al.,* 2012).

Studies to determine the microbial load of untreated POME at different stages of decomposition shows some disparity in the microbial counts. These variations in the range of microbial populations might be due to several reasons such as nutrient, minerals, temperature, oxygen level, acidity, volume of wastewater. This is collaborated by Okereke *et al.* (2007) and Jeremiah *et al.* (2018). The high population of bacteria in the POME may be linked with contaminations from poor sanitation in the mills (Okechalu *et al.* 2011). Secondly it might also be due to the handling process and the existing environmental conditions in the mills. Ohimain *et al.* (2013) stated that POME is a possible habitat for lipolytic and cellulolytic bacteria and fungi because it is rich in nutrients such as lipids and cellulosic materials. They isolated *A. fumigatus* and *A. niger* from POME sample and revealed that these microorganisms are capable of biodegradation of oily wastewaters. In this study, A. *terreus and A. nigger* were isolated from all the days except for A and E. *A. terreus* and *A. niger* have been well-known for its capability to survive in oily wastewater (Jeremiah *et al.* 2018). According to Ibiene (2011), *A. niger* can be grown in POME. This suggests the presence of the organism in all the samples tested. Its presence in the POME is also in line with the findings of Jeremiah *et al.* (2018). *A. terrus* was isolated from samples B, C and D. The utilization of hydrocarbon by different species of *Aspergillius* and some fungi has been reported by Obire *et al.,* (2008) and Ibiene *et al.,* (2011). According to Wong *et al.,* (2008), *Aspergilius* sp produces cellulose, the enzyme responsible for the breakdown of cellulase in POME.

Studies conducted by Kanokrat *et al.* (2013) revealed that some bacteria including Pseudomonas sp were isolated from palm oil contaminated soils had the ability to produce biosurfactants. Biosurfactants are amphiphilic surface-active agents produced by microorganisms that have the ability to reduce surface and interfacial tensions of fluids (Nitschke and Coast, 2007; Kanokrat *et al.,* 2013). As integral part of a biodegrading process, biosurfactants are effective in facilitating desorption of pollutants from the soil (Bustamante *et al.* 2012).

According to Okwute and Ijah (2014), *P. aeruginosa* is naturally associated with the degradation of palm oil and palm oil bearing materials. The occurrence of *P. aeruginosa* may be due to their ability to utilize oil as their carbon source. Pseudomonas are known to be capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants when grown on carbon source (Okwute and Ijah 2014). *Flavobacterium* sp was isolated in all the samples except on the 7th day (B). *Flavobacterium* sp has been shown to be associated with soil, dust, water and animals. The most common source of the organism in POME could be through handling and soil contamination during oil extraction. *Flavobacterium* are not only normal flora of the soil but can reproduce at the expense of gases produced from oil droplets.

Cellulomonas sp was isolated from samples B, C, D and E. The presence of these organisms in all the POME samples is indicative that the organism is associated naturally with POME.

Micrococcus luteus was isolated from sample A, C, D and E. Their presence in all these samples may be connected with their ability to degrade oil at low pH. (Jeremiah *et al.* 2018).

Phoma glomerate was isolated from samples A and E. The absence of the organism in sample B and C might be due to unfavorable growth conditions. *P. glomerate* are saprophytes which are unable to grow extensively until the onset of senescence. Their absence in B, C and E suggested that these organisms may not be part of the normal flora of POME.

Rhizopus stolonifer was isolated from all the samples except B. *Rhizopus stolonifer* is a mesophilic fungus. Studies have shown that *R. stolonifer* are associated with POME (Jeremiah *et al.* 2018). *R. stolonifer* is also a natural flora of the soil, and its presence in samples C and D might be due to contamination from red soil

DOI: 10.6092/issn.2281-4485/11667

used traditionally in the extraction. Studies have also shown that *Rhizopus* is involved in the biodegradation of plant materials. A seven-fold dilution of POME supported the growth of the organism.

Mucor racemosus was isolated from all the samples. *M. racemosus* has been associated with the degradation of plant and animal materials Obire *et al.,* (2008). The organism has also been isolated from palm oil fruits and other palm oil products.

Yeast cells were isolated from all the samples except A. The utilization of hydrocarbons by yeast especially *Candid* sp has been reported also by Obire *et al.,* (2008). *Brethanomyces internmedius* was isolated from sample E only. Its absence in samples C and D could be due to unfavorable growth conditions. Just like most opportunistic fungi, its growth in sample E might be due to presence of favorable growth conditions. Dormancy is a typical condition of fungi and some have been shown to remain dormant in the absence of available substrate and favorable growth conditions. This might explain the absence of the organism in all the samples except E. *Cladosporum* sp was isolated from samples A, D, and E. Studies have shown that *Cladosporum* is involved in the degradation of liquid waste (Omotayo *et al*., 2011; Okwute and Ijah 2014). This might explain the presence of the organism in samples D and E.

Variation in bacteria enumeration

Figure 1 shows the difference in bacterial load at different stages of decomposition. The results revealed a significant mean variation in microbial load in sample D (28 days after discharge) when compared with other samples. This is followed by a significant decrease in microbial load after 36 days of discharge, suggesting a depletion in organic content and nutrient availability.

Conclusion

This study demostrates that the microbial load of POME at various stages of degradation varies. It further revealed that the variation is proprtional to time and depends on the availability of organic matters present in the POME.The studies showed that numerous microorganisms are capable of growing and degrading POME. This study further shows that since POME has high BOD, COD and contains relatively many pathogenic microorganisms, it is therefore capable of polluting land and fresh water and should be allowed to undergo complete decomposition before discharge.

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DOI: 10.6092/issn.2281-4485/11667

Charge microbienne et biodégradation des effluents des huileries de palme (POME) par des microorganismes à différents stades de rejet

Résumé

L'effluent du moulin à huile de palme brute (POME) a une teneur extrêmement élevée en matières organiques dégradables en raison de l'huile de palme non récupérée lors de la production d'huile de palme. Le but de cette étude est d'évaluer la charge microbienne et la biodégradation du POME par des microorganismes à différents stades de décharge. Des méthodes de dilution standard ont été utilisées pour l'isolement des bactéries et des champignons. L'estimation de l'unité formant colonie par mL (cfu / mL) a été évaluée en utilisant la méthode de dilution en série 10 fois. Les résultats ont montré que la numération microbienne totale (TMC) diminuait avec le temps. L'analyse statistique utilisant l'analyse de la variance (ANOVA) a en outre révélé une variation significative de la charge microbienne après 28 jours de décharge. Cela a été suivi d'une diminution significative après 36 jours par rapport aux autres échantillons. Les études physico-chimiques ont en outre révélé la présence de niveaux relativement faibles de sodium, potassium, calcium, magnésium et fer. La concentration de nitrate était relativement élevée, 70,13 mg / L. La demande biologique en oxygène (DBO) et la demande chimique en oxygène (DCO) étaient également élevées respectivement de 2250 mg / L et 3360 mg / L. Des valeurs élevées de DBO et de DCO sont des indicateurs importants de composants organiques et inorganiques élevés de l'effluent et de possibles conséquences négatives sur l'environnement. La présence des espèces suivantes Clostridium, Proteus, Pseudomonas et Micrococcus impliquait une possible dégradation de l'effluent.

Mots clés: *moulin à huile de palme, effluent, pollution, charge microbienne, biodégradation*

Carica microbica e biodegradazione degli effluenti del frantoio di palma (POME) da parte di microrganismi a diversi stadi di scarico

Riassunto

L'effluente del frantoio per olio di palma grezzo (POME) ha un contenuto estremamente elevato di sostanze organiche declassabili come risultato dell'olio di palma non recuperato durante la produzione di olio di palma. Lo scopo di questo studio è valutare la carica microbica e la biodegradazione della POME da parte di microrganismi in diversi stadi di scarica. Sono stati utilizzati metodi di diluizione standard per l'isolamento di batteri e funghi. La stima dell'unità formante colonie per mL (ufc / mL) è stata valutata utilizzando un metodo di diluizione seriale 10 volte. I risultati hanno mostrato che la conta microbica totale (TMC) è diminuita con il tempo. L'analisi statistica che utilizza l'analisi della varianza (ANOVA) ha inoltre rivelato una variazione significativa della carica microbica dopo 28 giorni di dimissione. Questo è stato seguito da una significativa diminuzione dopo 36 giorni rispetto ad altri campioni. Gli studi fisico-chimici hanno ulteriormente rivelato la presenza di livelli relativamente bassi di sodio, potassio, calcio, magnesio e ferro. La concentrazione di nitrato era relativamente alta, 70,13 mg / L. Anche la domanda biologica di ossigeno (BOD) e la richiesta chimica di ossigeno (COD) erano elevate rispettivamente di 2250 mg / L e 3360 mg / L. Valori elevati di BOD e COD sono indicatori importanti di elevate componenti organiche e inorganiche degli effluenti e di possibili conseguenze ambientali negative. La presenza delle seguenti specie Clostridium, Proteus, Pseudomonas e Micrococcus implicava una possibile degradazione dell'effluente.

Parole chiave: *frantoio di palma, effluente, inquinamento, carica microbica, biodegradazione*