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Palm Oil Mill Effluent Biodegradation Potentials among Local Isolated Microorganisms

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ABSTRACT

The research study was aimed at investigating palm oil mill biodegradation potentials among locally isolated microorganisms. Palm Oil Mill Effluent (POME) polluted soil samples (0-15cm and 15-30cm depth) were collected into sterile polythene bags from Uruk Etta Ikot in Etim Ekpo L.G.A and Eyop industries at Akamkpa L.G.A, while POME wastewater was collected from Mfamosing at Akamkpa L.G.A, into glass containers with Teflon lined cap. The samples were placed in an ice-cool chest and transported to the laboratory for analysis. The study was completed within a duration of six months. Standard microbiological techniques were used to isolate, characterize and identify isolates from the collected samples. The biodegradation potentials of the isolates were adjudged based on their ability to reduce Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Oil and grease and Total Suspended Solids (TSS) of the palm oil mill effluents. The results obtained from the study showed that the BOD of the raw POME samples were reduced from 45,000±3.44mg/l to 37000±2.57mg/l (*Bacillus spp*), 36100±2.77mg/l (*Micrococcus spp*), 35,100mg/l (*Pseudomonas spp*), 35,200±3.08mg/l (*Aspergillus spp*), 33,400±2.54mg/l (*Fusarium spp*) and 25,600±1.81mg/l (*Mucor spp*). However the COD, Oil and grease, and TSS reduction potentials of the isolates followed similar pattern, with *Pseudomonas spp* (29,000±1.87mg/l), showing the highest COD reduction potential and *Mucor spp* (1,800±0.27mg/l, and 11,121±1.41mg/L) showing the highest oil and grease and TSS reduction potential respectively after a duration of 5 days. However, from this study, it is a clear indicator that *Pseudomonas spp* (with 53.33% BOD and 47.37% COD reduction efficiency), *Aeromonas spp*, (57.24% oil and grease reduction potential). *Fusarium spp* and *Mucor spp* (with 64% oil and grease reduction potential) and *Mucor spp* (23.20% TSS reduction potential) could be useful in enhancing the bioremediation and treatment of POME polluted environments.

INTRODUCTION

Palm oil industry has become one of the main agro-industries in Nigeria. Palm oil mills release POME in colossal amounts with its attendant polluting impending (Mohammadreza *et al.,* 2014). POME has unfavorable environmental ramifications effects including land and aquatic ecosystem contamination, loss of biodiversity and increase in COD and BOD in environment (Wu *et al.,* 2010). Today, the penetration of palm oil has been considered due to the entry of its effluents into the waterways and ecosystems, making it a meticulous concern towards the food chain interference and water consumption (Foo and Hameed *et al.,* 2010). This can cause considerable environmental problems such as land pollution and effectively suffocating aquatic life if discharged without effective treatment (Cheng *et al.,* 2010). In the process of palm oil milling, POME is mainly generated from sterilization and clarification of palm oil, in which a large amount of steam and hot water are used (Ohimain *et al.,* 2010). POME is a thick brownish liquid that is compiled with high concentrations of total solids, oil and grease, chemical oxygen demand (COD) and biological oxygen demand (BOD) (Neoh *et al.,* 2013). The biological treatment of POME depends enormously on consortium of microbial activities, which operate on the organic substrates present in the POME as supplements and eventually degrade these organic matters into simple by product such as methane, carbon dioxide hydrogen and water (Mohammed *et al.,* 2014). A variety of microorganisms have been investigated to be capable of biodegrading oil wastewater with high profits. Although, the anaerobic and aerobic treatment are one of the most sort biological methods for POME treatment. However the suspended and colloidal components are neither effectively decomposed biologically nor by other conventional means because they float the surface of wastewater and this the major problem to cause failure of treatment system (Ahmad *et al.,* 2011). However, with due consideration of the aforementioned, this study intends to investigate POME biodegradation potentials among locally isolated microorganisms.

MATERIALS AND METHODS

POME polluted soil samples:

POME polluted soil samples for this study was obtained from Uruk Etta Ikot in Etim Ekpo L.G.A and Eyop industries at Akamkpa L.G.A. The site was chosen with the intent of using soil samples that probably had in the past been exposed to POME.

Collection:

Surface soil (0 to 15cm depth) and subsurface soil (15cm-30cm depth) were collected using a Dutch auger. Collection was made from 3 to 4 random point, then bulked to form a composite sample and deposited in sterile labeled polyethylene bag and transported to the laboratory for further analysis.

POME wastewater

POME wastewater was collected into glass containers with Teflon lined cap from Eyop industries and Mfamosing, both located at Akamkpa L.G.A. The samples were placed in an ice-cool chest and transported to laboratory for analysis.

Media:

The media used in the study were; nutrient agar (Oxoid Ltd.), Sabouraud Dextrose Agar (Oxoid Ltd.) and Tributyrin agar (Oxoid Ltd.). The media were prepared in accordance to the manufacture instructions.

METHODS

Isolation of isolates

The method used was that of (Antai *et al.*, 2016). In this method, surface- spreading technique was used. Ten fold serial dilution of the POME polluted soil samples and wastewater samples were prepared. 0.1ml of 10^{-6} dilution was plated into nutrient agar and sabouraud dextrose agar plates. The nutrient agar plates were supplemented with 50µgml^{-1} of nystatin so as to inhibit fungal growth whole the sabouraud dextrose agar plates were supplemented with100µml^{-1} of streptomycin, so as to inhibit bacterial growth. The plates were prepared in duplicates and incubated at 37ºC for 24hours (nutrient agar plate) and 28ºC for 72 hours (sabouraud dextrose agar plate).

Purification and maintenance of microbial isolates

The bacterial and fungal isolates obtained from the nutrient agar and sabouraud dextrose agar plates were purified by repeated sub-culturing. The isolates were subjected to series of transfer into newly prepared medium. The bacteria isolates were transferred into newly prepared nutrient agar medium and incubated at 37ºC for 24 hours. The fungal isolates,

however, were transferred into newly prepared sabouraud dextrose agar and incubated at 28ºC for 3 days. Pure colonies of the bacteria and fungi were maintained on slopes of nutrient agar and sabouraud dextrose agar slant and stored in a refrigerator at 8ºC.

Characterization and identification of the isolates

Standard inocula were prepared from the preserved stock culture by taking a loopful of the bacterial and fungal isolates and aseptically inoculating onto sterile nutrient agar and sabouraud dextrose agar respectively. The plates were incubated at 28ºC for 24 hours. The characterization of the isolates was then performed by employing gram staining reaction, biochemical characterization and microscopic examination (Holt *et al.*, 1994; Cheebrough 2000; Hunter and Bennet, 1993).

Biodegradation test

250ml of raw palm oil mill effluent was introduced into conical flash and sterilized at 121ºC for 20 minutes. The sterilized raw palm oil mill effluent was allowed to cool before inoculation. 20mls of broth sample containing the inoculums was used to inoculate 250mls of the palm oil mil effluent samples and was shaken and incubated at room temperature for 24 hours (bacteria) and 3-4 days (fungi). The samples were aseptically drawn for 5 days and analyzed for BOD, COD, oil and grease and TSS, with the control flasks not inoculated. The percentage reduction was measured by using the equation as described by (Jeremiah *et al.*, 2014).

$$\text{Reduction (\%)} = \frac{Craw\ POME - Cf}{Craw\ POME} \times \frac{100}{1}$$

Where;

Craw POME is the concentration of COD, BOD, TSS, OIL and grease of raw POME and cf is the concentration of these parameters after treatment.

RESULTS

Palm oil mill effluent biodegradation potential by isolates from collection sample.

Table 1 presents result of POME biodegradation potential of selected isolates from collected samples. It showed that *Bacillus spp* (PCE_1) was able to reduce the BOD of the raw POME

from 45,000 2.87mg/l to 37,000±2.87mg/l after a duration of 5 days reduced while BOD value of 36,100±2.77mg/l, 22,100±1.57mg/l, 29,700±2.18mg/l, 35,600±1.81mg/l were obtained from *Micrococcus spp* (PSC_1), *Pseudomonas spp* (PSC_3), *Aeromonas spp* (PSC_6), *Geotricum spp* (PSU_{18}), *Aspergillus spp* (PSC_5), *Fusarium spp* (PSC_{18}) and *Mucor spp* (PUE_6) respectively (fig 1). The COD of the POME was reduced from 55,100±3.89 to 40,501±3.21mg/l (*Bacillus spp*), 46,123±3.14mg/l (*Micrococcus spp*), 29,000±1.87mg/l (*Pseudomonas spp*), 40,100±3.15mg/l (*Aeromonas spp*), 45,200±2.56mg/l (*Geotricum spp*) 44,200 ±2.98 (*Aspergillus spp*), 43,100±2.89mg/l (*Fusarium spp*) and 38,400±2.05mg/l (*Mucor spp*) (fig 2). However, the oil and grease and TSS of the palm oil mill effluent substrate followed similar pattern (fig 3 and 4).

Table 1: Palm oil mill effluent biodegradation potential of isolated isolates from collected samples

Isolate code	Number of days	BOD(mg/L)	COD(mg/L)	Oil and Grease(mg/L)	TSS(mg/L)	Probable organism
PCE_1	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Bacillus spp*
	2	40,300± 2.88	37,470± 2.43	4,560± 1.72	14,300± 2.48	
	3	38,700± 2.30	48,000± 3.55	4,100± 1.68	13,800± 2.35	
	4	41,500± 3.22	46,700± 3.43	4,000± 1.60	13,200± 2.25	
	5	37,400± 2.87	40,501± 3.21	3,800± 1.51	12,900± 2.15	
PSC_1	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Micrococcus spp*
	2	38,210± 2.30	50,300± 3.62	4,900± 1.78	14,100± 2.38	
	3	36,500± 2.28	51,105± 3.67	3,700± 1.48	13,900± 2.28	
	4	39,301± 2.88	48,000± 3.55	3,520± 1.33	13,540± 2.18	
	5	36,100± 2.27	46,123± 3.41	3,300± 1.28	12,000± 1.97	
PSC_3	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Pseudomonas spp*
	2	35,200± 2.18	35,190± 2.33	3,400± 1.32	14,101± 2.39	
	3	28,800± 1.97	33,000± 2.15	3,180± 1.28	14,068± 2.31	
	4	25,240± 1.66	30,100± 1.98	2,190± 1.23	13,780± 2.11	
	5	21,000± 1.57	29,000± 1.87	1,900± 1.01	13,660± 2.08	
PSC_6	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Aeromonas spp*
	2	28,800± 1.95	42,200± 3.17	3,200± 1.31	14,098± 2.37	
	3	24,200± 1.59	39,420± 2.11	3,100± 1.11	14,053± 2.27	
	4	30,500± 2.33	37,260± 1.96	3,080± 0.98	13,590± 2.17	
	5	29,700± 2.18	40,100± 3.15	2,138± 0.77	12,300± 2.13	

PSU_{18}	1	45,200± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Geotricum spp*
	2	38,700± 2.39	53,200± 3.29	4,000± 1.68	13,121± 2.12	
	3	36,100± 2.28	52,600± 3.15	3,800± 1.58	13,011± 2.11	
	4	33,200± 3.11	48,100± 2.88	3,420± 1.38	12,281± 1.98	
	5	35,100± 3.60	45,200±2.56	3,200± 1.21	12,211± 1.95	
PCE_5	1	45,300± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Aspergillus spp*
	2	44,100± 3.32	52,300± 3.67	3,600± 1.11	14,112± 2.45	
	3	41,300± 3.18	49,300± 3.15	3,400± 1.21	14,001± 2.35	
	4	38,400± 3.17	49,100± 3.01	3,200± 1.18	13,411± 2.25	
	5	35,200± 3.08	44,200±2.98	3,100± 1.08	13,010± 2.11	
PSC_{18}	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Fusarium spp*
	2	38,700± 2.39	49,100± 3.12	2,600± 0.78	13,512± 2.12	
	3	36,800± 2.92	47,600± 2.88	2,500± 0.68	13,311± 2.11	
	4	37,600± 2.98	45,400± 2.39	2,320± 0.54	12,611± 1.98	
	5	33,400± 3.60	43,100±2.89	2,800± 1.21	12,302± 1.88	
PUE_6	1	45,000± 3.44	55,100± 3.89	5,000± 1.88	14,480± 2.55	*Mucor spp*
	2	36,200± 2.91	44,200± 2.98	4,000± 0.37	12,781± 1.98	
	3	32,300± 2.94	43,000± 2.58	2,200± 0.27	11,821± 1.55	
	4	28,400± 1.87	41,600± 2.35	1980± 0.29	11,412± 1.45	
	5	25,600± 1.81	38,400±2.05	1,800± 0.27	11,121± 1.41	

Keys; BOD= Biological oxygen demand, COD= Chemical oxygen demand, TSS= Total suspended solids.

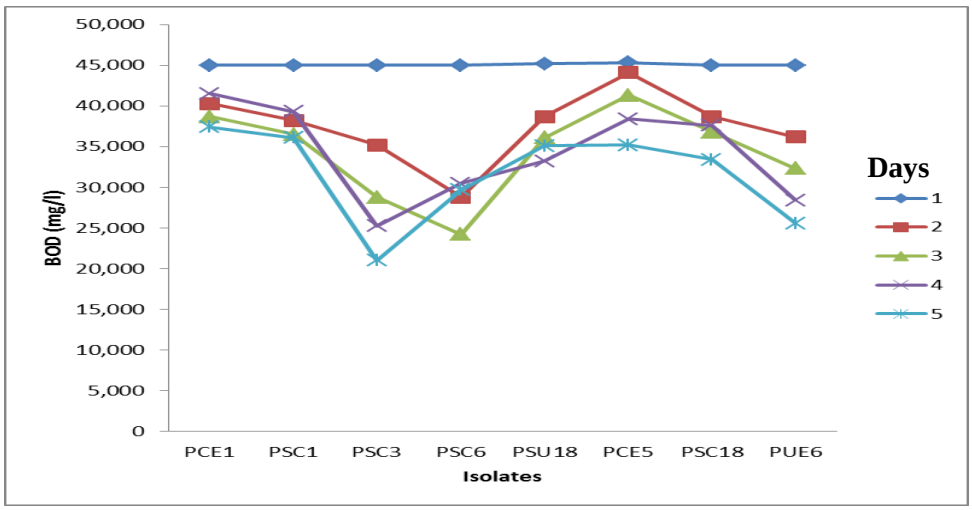

Fig 1: BOD reduction by isolates from samples

Key: PCE_1: *Bacillus spp* PSC_1: *Micrococcus spp* PSC_3: *Pseudomonas spp* PSC_6: *Aeromonas spp* PSU_{18}: *Geotricum spp* PCE_5: *Aspergillus spp* PSC_{18}: *Fusarium spp* PUE_6: *Mucor spp*

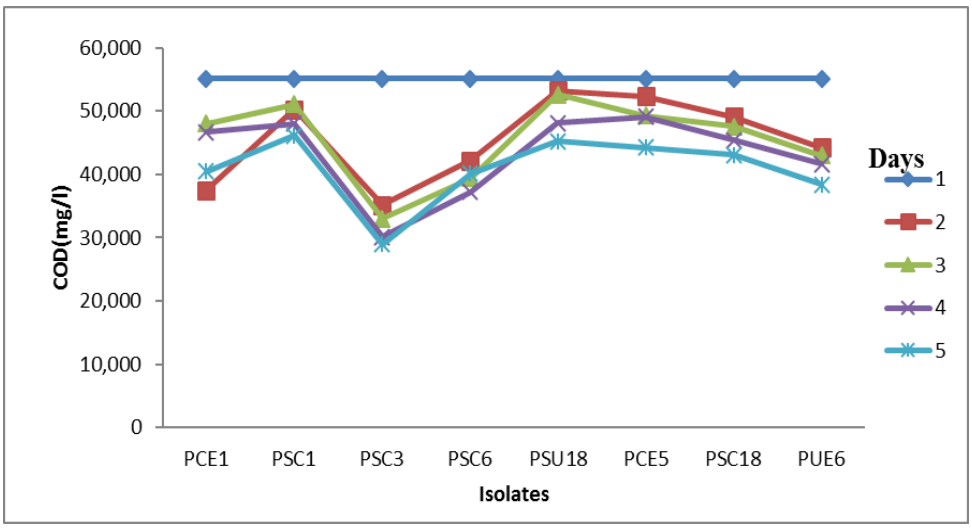

Fig 2: COD reduction by isolates from samples

Key: PCE_1: *Bacillus spp* PSC_1: *Micrococcus spp* PSC_3: *Pseudomonas spp* PSC_6: *Aeromonas spp* PSU_{18}: *Geotricum spp* PCE_5: *Aspergillus spp* PSC_{18}: *Fusarium spp* PUE_6: *Mucor spp*

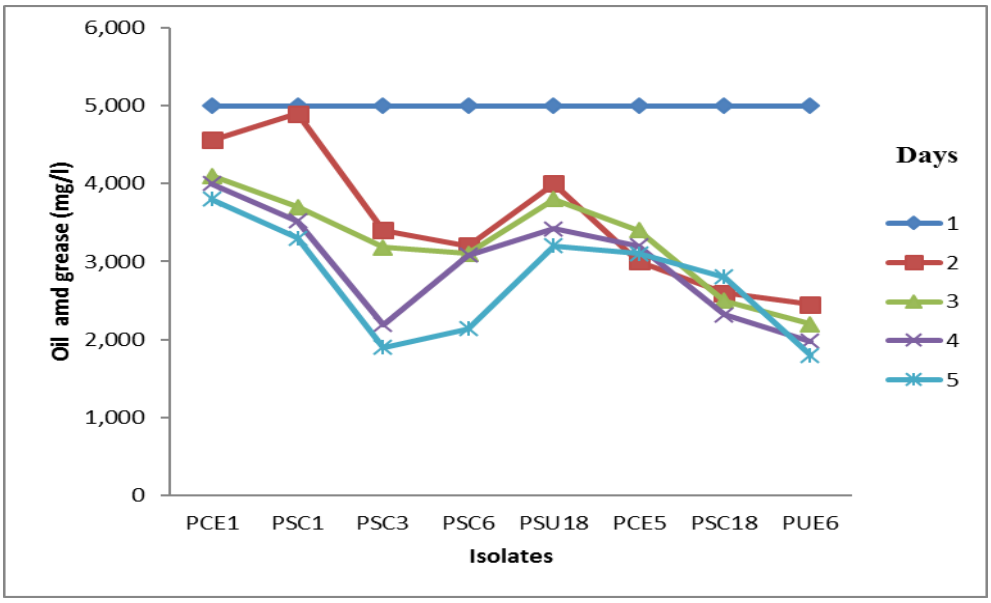

Fig 3: Oil and grease reduction by isolates from samples

Key: PCE_1: *Bacillus spp* PSC_1: *Micrococcus spp* PSC_3: *Pseudomonas spp* PSC_6: *Aeromonas spp* PSU_{18}: *Geotricum spp* PCE_5: *Aspergillus spp* PSC_{18}: *Fusarium spp* PUE_6: *Mucor spp*

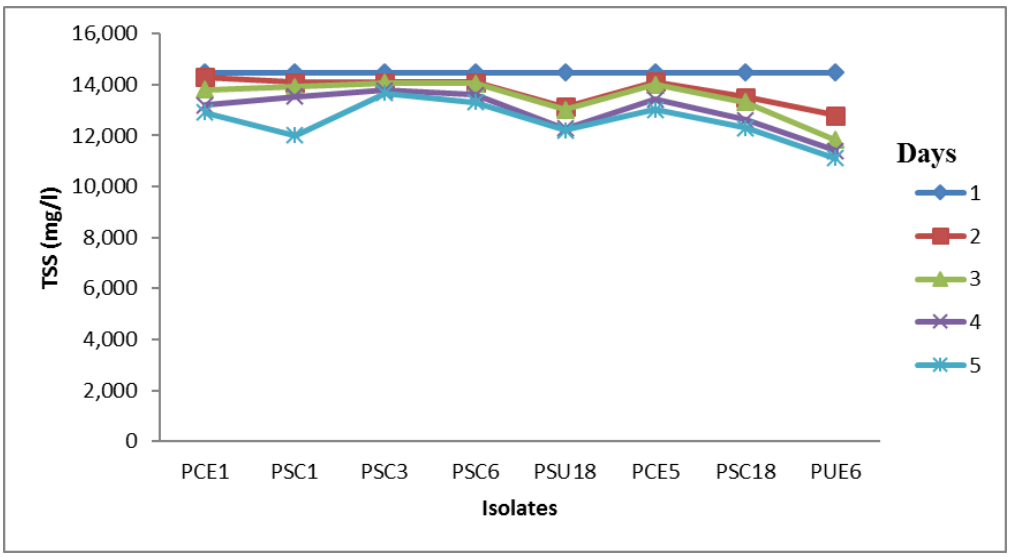

Fig 4: TSS reduction by isolates from samples

Key: PCE_1: *Bacillus spp* PSC_1: *Micrococcus spp* PSC_3: *Pseudomonas spp* PSC_6: *Aeromonas spp* PSU_{18}: *Geotricum spp* PCE_5: *Aspergillus spp* PSC_{18}: *Fusarium spp* PUE_6: *Mucor spp*

Palm oil mill effluent percentage reduction potential of selected isolates from samples

Table 2 presents the result of palm oil mill effluent percentage reduction potentials of selected isolates from samples. It showed that *Pseudomonas spp* (PSC_3) had the highest BOP percentage reduction (53.33%) (fig 5), and COD percentage reduction (47.37%) (fig 6). *Mucor spp* (PUE_6) and *Fusarium spp* (PSC_{18}) had the highest oil and grease percentage reduction (64%) (fig 7) while *Mucor spp* (PUE_6) had the highest TSS percentage reduction (23.20%) compared to other isolates (fig 8)

Table 2: Palm oil mil effluent percentage reduction potential of selected isolates from samples

Isolate code	BOD (%)	COD (%)	Oil and Grease (%)	TSS (%)	Probable organism
PCE_1	16.87	26.49	24	10.91	*Bacillus spp*
PSC_1	19.78	16.29	34	17.12	*Micrococcus spp*
PSC_3***	53.33	47.37	62	5.66	*Pseudomonas spp*
PSC_6***	34	27.22	57.24	8.15	*Aeromonas spp*
PSU_{18}	22.35	17.82	36	15.67	*Geotricum spp*
PCE_5	22.30	19.78	44	15.04	*Aspergillus spp*
PSC_{18}***	25.78	21.78	64	15.04	*Fusarium spp*
PUE_6***	43.11	30.31	64	23.20	*Mucor sspp*

Keys: ***= Potential microorganisms for biodegradation of palm oil mill effluents, TSS= Total suspended solid, BOD= Biological oxygen demand.

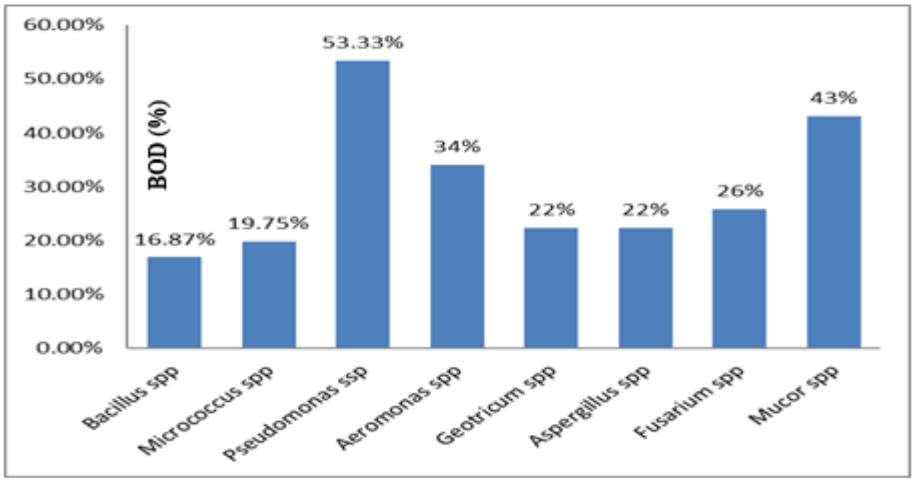

Fig 5: BOD percentage reduction potential of selected isolates from samples

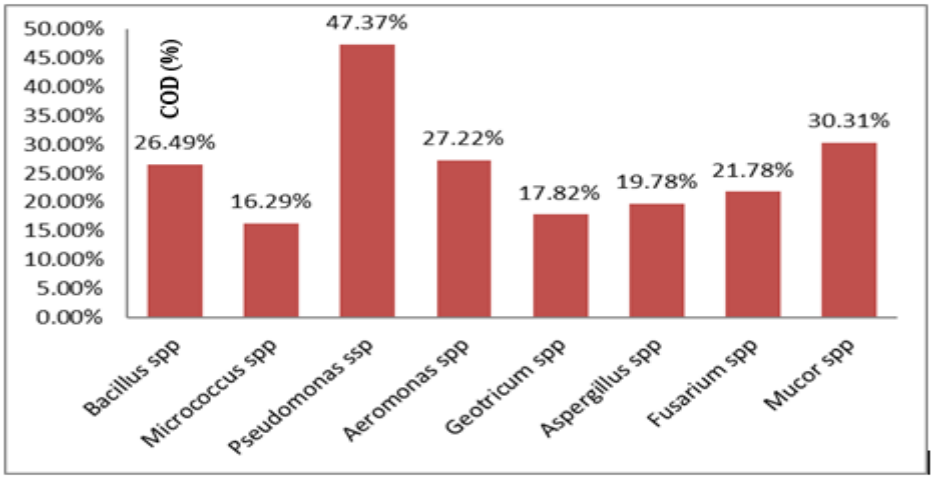

Fig 6: COD percentage reduction potential of selected isolates from samples

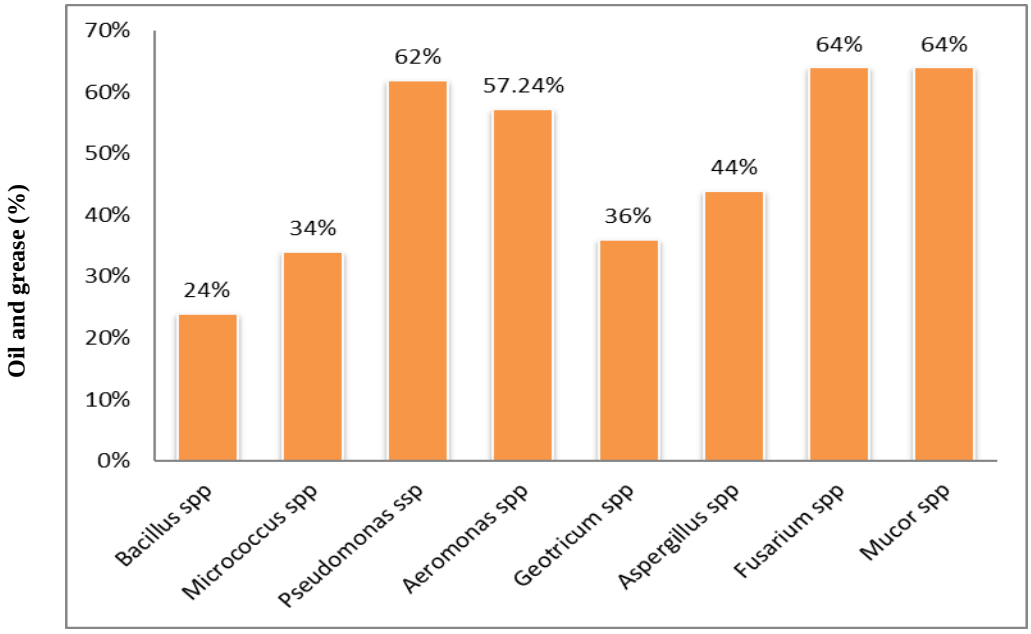

Fig 7: Oil and grease percentage reduction potential of selected isolates from samples

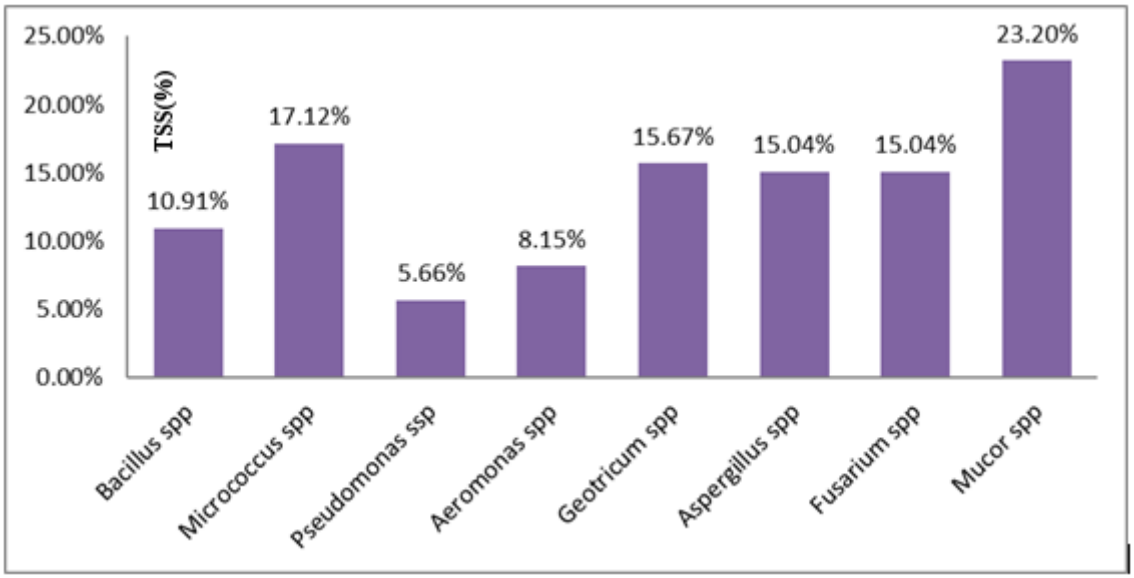

Fig 8: TSS percentage reduction potential of selected isolates from samples

DISCUSSION

Palm mill effluent microorganisms have been known to survive in only wastewater or soils by producing the enzyme lipase and or spores (as the case may be with fungi) which enables them in surviving the anaerobic nature of POME (Ohimain *et al.*, 2012). Orj*i et al.*, (2006) stated that oily substrate provides a good environment for lipolytic microorganisms to flourish. The biodegradation of oil in the environment is a complex process, whose

quantitative and qualitative aspects depend on nature and amount of the oil present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community (leachy and Colwell, 1990; Hinchee and Olfenbuttel, *et al.*, 1991). This is in compliance with this study, whereby constitution of in POME (Izah and Ohimain. 2013). (Orji *et al*., 2006) stated that oily substrate provides a good environment for lipolytic microorganisms to flourish. This is in compliance with this study, whereby constitution of indigenous bacteria and fungi isolated from POME and its polluted soil samples were used for degradation of palm oil mill effluent samples. The ability of the bacterial and fungi isolates to biodegrade palm oil mill effluent was demonstrated in terms of reduction in BOD (mg/L), COD (mg/L), TSS (mg/L), Oil and Grease(mg/L) and percentage degradation determined from the equation earlier mentioned.

Amongst, the bacteria isolates screened for palm oil mill effluent biodegradability for 5 days, *Pseudomonas spp* (PSC_3) showed the highest BOD (53.33%) and COD (47.37%) while *Fusarium spp* (PSC_{18}) and *Mucor spp* (PUE_6) showed the highest Oil and grease (64% each) and *Mucor spp* (PUE_6) had the highest TSS reduction potential (23.20%). In compliance with the observation from this study, other researchers have reported biodegradation of oily wastewaters with significant reduction in TSS, Oil and Grease and similar parameters (El-masery *et al*., 2005; Raj and Murthy, 1999).

Generally, the BOD, COD, TSS and Oil and grease reduction observed is as a result of microbial oil degradation which is considered to occur as a result of hydrolysis of oil by secretion of lipase (oil degradation enzyme), which degrades the oil to organic acids and Volatile Fatty Acids (VFA) or reduces it to a low molecule *via* beta oxidation (Fatty acid degradation pathway) and finally the oil is decomposed to CO_2 and H_20 (Koshimizu *et al*., 2007). Since Palm oil mill effluent contains a very high organic matter (Organic carbon) (as evident in the result of Physicochemical analysis obtained in this study) which is generally biodegradable, this facilitates the application of biological treatment based on aerobic process (Chin and Wong *et al*., 2003). This biological treatment depends greatly on active microorganisms, which utilizes the organic substances present in the POME as nutrients and eventually degrades these organic matters into simple by-products such as methane, carbondioxide, hydrogen sulphide and water (Jameel and Olanrewaju *et al*., 2011).

Thus, the exploitation of these microorganisms isolated from palm oil mill effluent for biodegradation and bioremediation purposes will offer a very efficient tool for purifying contaminated effluents water.

CONCLUSION

From this study, it is evident that POME degrading microorganisms can be isolated from POME polluted area and the degrading ability of these microorganisms; *Pseudomonas spp* (PSC_3), *Aeromonas spp* (PSC_6), *Fusarium spp* (PSC_{18}), *Mucor spp* (PUE_6) is a clear indicator that they can be applied in bioremediation techniques for biodegradation of POME to enhance treatment.

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