British Microbiology Research Journal
4(12): 1440-1450, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Biodegradation of Palm Oil Mill Effluent

P. N Ibegbulam-Njoku[1*] and O. K. Achi[1]

[1]Microbiology Department of Michael Okpara University of Agriculture, Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author PNIN participated in all operations of this manuscript. Author PNIN wrote the first draft of the manuscript. Authors OKA designed the study and wrote the protocol performed. Author PNIN managed the analyses of the study and managed the literature searches. Author OKA revised the manuscript and author PNIN has the final responsible for all information presented. Both authors read and approved the final manuscript.

Original Research Article

Received 13th June 2014
Accepted 17th July 2014
Published 3rd August 2014

ABSTRACT

Aims: The aim of this study was to investigate the biodegradation capacity of selected indigenous fungal isolates and optimization of their degradation ability using various environmental factors such as pH, incubation temperature, nutrient concentration and inoculums size in reducing pollution effect of palm oil mill effluent (POME) in the environment.
Place and Duration of Study: Two fungal isolates *Candida rugosa* and *Geotrichum candidum* used in this work were previously isolated from POME sample collected from Starline palm oil mill industries, Umukalika, Obingwa LGA, Abia state Nigeria in previous work of authors. The study was carried out from March to August, 2013.
Methodology: Spore suspension was prepared by adding 10 ml of 0.1% Tween 80 onto PDA slant of 5 days old culture of *Candida rugosa* and *Geotrichum candidum* respectively. Biodegradation of POME was carried out by inoculating 0.1ml (10^6spores/ml) of respective fungal isolates into different 500 ml Erlenmeyer flasks containing 100ml each of raw POME. They were incubated at 30ºC on a rotary shaker (200rpm). Samples were taken every 24hrs for 144hrs to determine BOD, COD, oil & grease. Similarly, optimization of biodegradation was carried out by studying the effect of different environmental conditions such as different initial pH levels (4.0-8.0), incubation temperature (25-50ºC), concentrations of soy bean (1.5-4.5% w/v) and inoculum size (0.1-0.5 v/v). The experiments were done in triplicates.
Results: Biodegradation studies with selected indigenous fungi showed that *C. rugosa*

**Corresponding author: Email: peacennem@gmail.com;*

was able to remove (44.6%) BOD, (13.9%) COD , (50.7%) oil and grease (O&G) while *G. candidum* reduced BOD, COD, O&G by 46.9%,16.9% and 64,9% respectively after 144hrs. Optimization of degradation in POME using various environmental and nutrients conditions revealed that at pH 8, *C. rugosa* showed best degradation of COD (48.6%), BOD (74.5%), O&G (41.8%) removal while COD (59.1%), BOD (75.7%) , O&G (59.1%) removal was observed with *G. candidum* treatment. The optimal incubation temperature for degradation using each of fugal isolates was at 35ºC with 85.2% BOD , 71.8% COD and 67.3% O&G removal for *C. rugosa* , 87.3% BOD and 63.4% COD for *G. candidum* .The best degradation ability for *C. rugosa* and *G. candidum* were demonstrated at 3.5w/v and 2.5w/v soybean concentrations respectively. The result also showed that increase in inoculum size could not completely reduce oil and grease during degradation process possibly because no single culture supports degradation optimally due to presence of complex sugars

Conclusion: The selected fungal isolates exhibited high efficiency for removal of oil and grease as well as organic matter from POME but required control of environmental conditions and nutrient expansion for the effective biodegradation of POME.

Keywords: Biodegradation; BOD; COD; oil and grease; Palm oil mill effluent.

1. INTRODUCTION

In recent years, attention has been drawn to environmental hazards caused due to discharge of industrial effluent. Some technologies have been developed to treat these industrial effluents so as to meet the Department of Environmental (DOE) discharge standard [1]. Establishment of palm oil industries by individual or cooperative societies in rural areas of southern Nigeria have provided source of livelihood to many families and employment opportunities to teaming youths in such local government areas. The rapid development of these industries, have also generated high level of wastewater known as palm oil mill effluent (POME) from palm oil milling activities.

POME is the largest by-product during the palm oil extraction with about 1.5 tones of POME being generated for a tone of fresh fruit bunch (FFB) processed [2]. It consists of high amount of solid and liquid wastes. POME is a thick brownish color liquid that contains high level of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids, fairly acidic pH , oil and grease [1,3].

In most cases, the untreated effluent is discharged either to nearby local palm plantation or surface water body around the area due to the cost of conventional wastewater treatment. It encourages coloration of water (black), eutrophication thereby supporting growth of water hyacinths also destabilizing the overall quality of drinking water to the inhabitants of such areas [4,5]. The highly polluting effect is becoming a serious problem to environment when discharged untreated into the environment based on WHO standard limit imposed .Considering this fact, biodegradation is preferred to most chemical treatments due to lower costs and the complete mineralization. However, controversy over whether to use natural or genetically engineered micro-organisms (GEM) in biodegradation is lingering in recent times. Government agencies are not willing to release GEMs into the environment due to its potential unpredicted ecological impact [6].

The biodegradation processes in POME are of great interest to many researchers [7-9] due to high level of organic matter which indicates high BOD and COD values as well as oil and grease which serve as substrates for microorganisms [8]. Oil in POME is an excellent source of carbon showing about twice the energy value of glucose during microbial growth [9].

Therefore, the use of fungi in biodegradation process provides an alternative to clean up environmental pollutants and have drawn interest in researches in the past two decades since most biodegradation researches focused mainly on the use of bacteria. The major interest in the use fungi is associated to their bioremediation potential due to the enzymes they produced that are mainly used in lignin breakdown and degrade various ranges of recalcitrant pollutants [10].

Various fungi have the ability to produce extracellular lipase enzymes that hydrolyze triglycerides to fatty acids and glycerol [11,12]. The production of lipases in fungi have been well studied such as *Rhizopus, Mucor, C. rugosa, G. candidum and Aspergillus* [13-16] as most of these fungal isolates can grow on vegetable oil sources, this trait makes it possible for their usage in biodegradation of POME hence this work is taken up.

The aim of this study was to investigate biodegradation capacity of selected indigenous fungal isolated and optimization of degradation ability using various the environmental factors (pH, incubation temperature, organic concentration and inoculums size) in reducing pollution effect of POME in the environment .

2. MATERIALS AND METHODS

2.1 Sample and Pure Cultures Collection

Two fungal isolates *C. rugosa* and *G. candidum* used in this work were previously isolated from POME sample collected from Starline palm oil mill industries Umukalika Obingwa Abia state Nigeria in previously work of author [17].

2.2 Sampling and Characterization of POME

Characterization of the POME was carried out before and after the treatment to determine the efficiency of the treatment. The samples were collected and analyzed using standard methods [18] in order to monitor the biodegradation process. The Biochemical Oxygen Demand (BOD) of the raw POME was determined using HACH model 2173 BOD measurement apparatus) while Chemical oxygen Demand (COD) was determined by titrimetic method as described in standard method for the Examination of water and wastewater [17] using Ferrous ammonium sulphate $(NH_4)_2SO_4$. $FeSO_4.6H_2O$. Oil and grease (O&G) determination was equally carried out using gravimetric method after soxhlet extraction [19].

2.2.1 Biodegradation of raw POME using fungal isolates

2.2.1.1 Spore suspension preparation

The method is as described by [20] with slight modification. Spore suspension was prepared by adding 10ml of 0.1% Tween 80 onto PDA slant of 5 days old culture of *C. rugosa* and *G. candidum* respectively.

2.2.2 Biodegradation

Each fungal isolate, *C. rugosa* and *G. candidum* of innoculum size of 0.1ml (10^6spores/ml) was introduced into 500ml Erlenmeyer flasks containing 100 ml each of raw POME. They were incubated at 30ºC on a rotary shaker (200rpm). Samples were taken every 24hrs for 144hrs to determine BOD, COD, O&G.

2.2.3 Optimization of biodegradation of POME

Different environmental conditions were used to study optimization of biodegradation of POME using the respective fungal isolates .At different initial pH levels (4.0-8.0) using method described by [21], effect of incubation temperature (25-50ºC) as described by [11] and effect of different concentrations of soy bean (1.5 - 4.5w/v) as described [22].

The respective fungal isolates innoculum size of 0.1ml (10^6spores/ml) was introduced into 100ml of POME for each of the specific parameters checked and appropriately adjusted .It was incubated for 144hrs at 30ºC. The biodegraded palm oil mill effluent samples were drawn and determined for growth as BOD, COD, O&G. Statistical analysis was conducted with SPSS program by Duncan's model at 0.05 levels.

2.2.4 Effect of inoculum size on biodegradation of POME

The method used was described by [23]. The effect of inoculum size on biodegradation of palm oil mill effluent was studied in the range of 0.1-0.5 v/v of the isolated fungi and incubated at 30ºC for 144hrs. Samples were drawn and tested for growth at BOD, COD and O&G.

The efficiency for organic load (BOD or COD) reduction was calculated as described by [11]

$$\frac{\text{Initial (BOD or COD) of the raw POME - final (BOD or COD) after treatment}}{\text{Initial (BOD or COD) of the raw POME}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Biodegradation of Raw POME using Isolated Fungi

The effluent used in this study was obtained from the sterilizer, separator during oil extraction and washing of the palm kernel. These processes result in the loss of oil and contribute a large percentage of the polluting organic load in the effluent [9]. Table 1 shows biodegradation of POME using isolated fungi with the control showing characteristics of raw POME with high organic matter of BOD 16280±25mg/L, COD values of 78750±41mg/L, oil & grease (5,165±08mg/L). After treatment of the POME with respective fungal isolates over a period of 144hrs, *C. rugosa* degraded the organic matter in the effluent with BOD reduction of 44.6%, COD 13.9%, O&G removal 50.7%. Similarly *G. candidum* degraded the POME with BOD of 46.9%, COD 16.9%, O&G 64.9% reduction. The reduction in the organic matter was synonymous with the O&G removal from the effluent. Since oil and grease contain triacylglycerols that are hydrolyzed by fungal lipases to yield diacylglycerol, monoacylglycerols, free fatty acids and glycerol the use of the selected fungi was very useful [9]. The high organic matter is usually associated with the presence of different sugars such as arabinose, xylose, glucose, galactose and mannose respectively [24]. However, since the

POME is said to be non-toxic as no chemical is added in the oil extraction process, it become good source of nutrients for microbial growth thereby reducing the COD and BOD)and increase organic nitrogen availability to plants within the surrounding where treated effluent is discharged [20,24]. Therefore, the results obtained indicated that treatment of POME before discharge would reduce the risk of waterway pollution.

3.2 Effect of pH on Biodegradation of POME Using Isolated Fungi

Five pH values from 4.0 to 8.0 were investigated (Table 2). POME was degraded rapidly at pH 8.0 when inoculated with the respective fungal isolates used in this study. The highest COD (48.6%), BOD (74.5%) with O&G (41.8%) was observed at pH 8 using *C. rugosa* in the biodegradation of POME while *G. candidum* also showed best degradation ability with COD(59.1%), BOD (75.7%), O&G (59.1%) removal also at pH 8. At pH 7 and 8, there was improvement on the organic load removal using the respective isolates. Similarly, the increase in pH improved removal of oil and grease. Previous studies have shown that microorganisms have Optimal pH of 6.8 to 7.2 for growth while both acidic pH of 4 and alkaline pH higher than 9.5 are not tolerable by most organisms [25]. The result improved pH of the POME for plant reutilize and discharge into river streams. It confirms previous report on the importance of treatment of acidic raw POME, since it affects nutrient availability of the nearby plants when discharged into the soil or stream [26]. Similarly, reports of accumulation of high volatile fatty acid concentration during effluent treatments and drop in pH have been said to inhibit methanogenesis especially during anaerobic degradation of oil related effluents [27-29]. However, other studies have improved this system (control of volatile fatty acid) by increasing the pH slightly using many factors such as addition of lime and bicarbonate salts [25,30].

3.3 Effect of Incubation Temperature on Biodegradation of POME using Isolated Fungi

The biodegradation performance of each of the selected fungal isolates was investigated considering various incubation temperature (25 to 50ºC) for 144hrs to determine the optimum incubation temperature for biodegradation of POME. The study was carried out using *C. rugosa* and *G. candidum* respectively. Table 3 revealed that the optimal incubation temperature of biodegradation for *C. rugosa* was at 35ºC with 85.2% BOD , 71.8% COD and 67.3% O&G removal , similarly *G. candidum* degraded POME at optimum incubation temperature of 35ºC with BOD (87.3%), COD (63.4%) and O&G removal of 58.8%. The Increase in temperature improved the rate of oil removal. The study supported the report of Zekri and Chaalal [31] who reported that increase in temperature encouraged biodegradation of crude oil by bacteria similarly Khleifat [32] also reported biodegradation of phenol by *Ewingella americana* at optimal temperature of 35-37ºC. However, Rekha et al. [33] found that the optimum lipase production in *C. rugosa* at 32ºC was (50.25U/ml) and suggested that increase in temperature affected lipase production in *C. rugosa*.

3.4 Effect of Soy Bean Concentration on Biodegradation of POME using Isolated Fungi

The effect of soybean concentration on the degradation of POME was demonstrated in Table 4 using different concentrations (1.5 to 4.5w/v). The organic nitrogen (soybean) showed good degradation capacity at 2.5-3.5w/v for using the selected fungal isolates. *C. rugosa* showed best biodegradation at concentration 3.5w/v with BOD and COD removal of 85 and 64.5% respectively while *G. candidum* showed highest degradation of 81% BOD and 46.2% COD removal at 2.5w/v of soybean. Previous studies on the use of organic nitrogen in the presence of olive oil for lipase production by *C. rugosa* revealed that nitrogen sources alone encouraged very high lipase activities [34].

3.2 Effect of Inoculum Size on Biodegradation of POME using Isolated Fungi

The study on the effect of inoculum size on biodegradation of POME using respective fungal isolates was carried out using 0.1 to 0.5v/v. The highest efficiency of oil and grease removal was observed as 43.6% with *C. rugosa* and 40% with *G. candidum* treatment. The BOD and COD reductions were best at 69.4% and 37.3% using inoculums size of 0.2v/v *C. rugosa* (Fig. 1) while *G. candidum* also showed best degradation activity at 0.2v/v inoculum size with 77.8% BOD and 39.9% COD reduction (Fig. 2). These results showed that increasing inoculum size could not reduce oil and grease during degradation process possibly because of succession process involved in biodegradation process as no single culture is expected to breakdown complex sugars associated with high organic matter. Bhumibhamon et al. [35] studied the effect of inoculum size with single culture and mixed cultures were it was discovered that increasing inoculum size could not improve fat degradation.

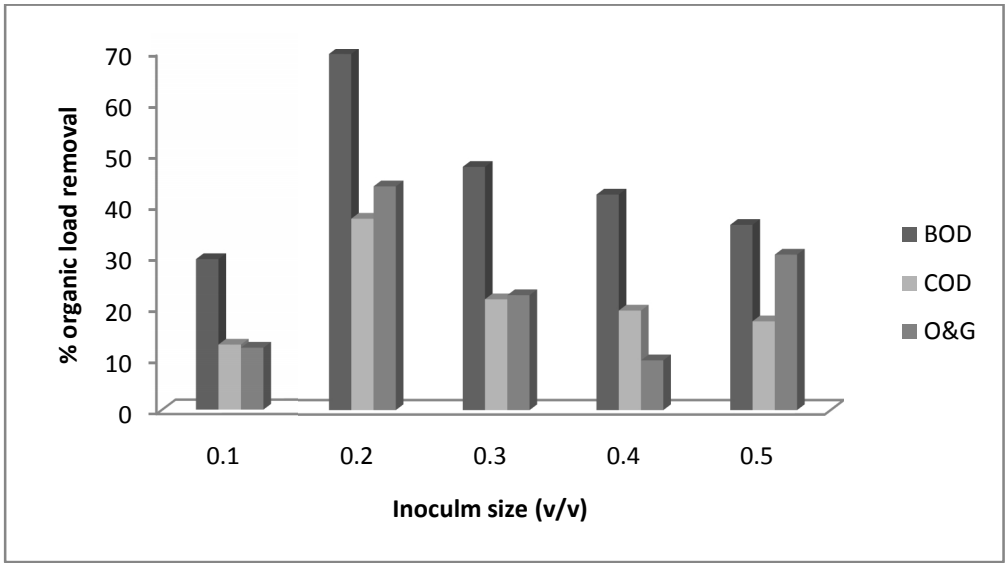

Fig. 1. Effect of inoculum size on biodegradation of POME using *C. rugosa*

Table 1. Biodegradation of POME using isolated fungi

Organism	Time (hrs)	BOD_5 (mg/L)	% Reduction	COD (mg/L)	% Reduction	O&G (mg/L)	% Reduction
Control	-	16280±25	-	78750±41	-	5165±08	-
C. rugosa	24	15840±17	2.7	77480±33	1.6	4963±04	3.9
	48	14140±08	13.1	75680±02	3.9	4160±41	19.5
	72	13004±15	20.1	72750±17	7.6	3657±13	29.2
	96	11980±42	26.4	70637±04	10.3	3005±16	41.8
	120	9634±04	40.8	69491±07	11.8	2948±15	42.9
	144	9024±12	44.6	67842 ±16	13.9	2548±04	50.7
G. candidum	24	15304±05	6.0	76480±31	2.9	5062±11	1.9
	48	14080±12	13.5	75240±09	4.5	4058±02	21.4
	72	12606±05	22.6	72586±23	7.8	3255±11	37
	96	11780±12	27.6	70075±14	11.0	2854±17	44.7
	120	9560±09	41.3	67290±12	14.6	2017±09	60.9
	144	8645±16	46.9	65428±02	16.9	1814±19	64.9

**Values are the means of replicate determinations ± SD*

Table 2. Effect of pH on biodegradation of POME using isolated fungi

Organism	Initial pH	BOD_5 (mg/L)	% Reduction	COD (mg/L)	% Reduction	O&G (mg/L)	% Reduction
Control	4.0	16280±25	-	78750±11	-	165±12	
C. rugosa	4.0	7934±67	51.2	50130±73	36.3	104±27	36.9
	5.0	6870±38	57.8	60500±72	23.2	121±31	26.7
	6.0	8840±55	45.7	67980±87	13.6	154±37	6.7
	7.0	5340±39	67.2	42807±47	45.6	84±14	49.1
	8.0	4150±13	74.5	405071±67	48.6	96±35	41.8
G. candidum	4.0	9950±37	38.8	65230±48	17.2	160±18	3.0
	5.0	5980±27	63.3	40720±72	48.3	78 ±17	52.7
	6.0	6206±25	61.8	46690±32	40.7	130±17	21.2
	7.0	4204±15	74.2	37150±47	52.8	147±19	10.9
	8.0	3960±39	75.7	32220±32	59.1	103±10	37.6

**Values are the means of replicate determinations ± SD*

Table 3. Effect of incubation temperature on biodegradation of POME using isolated fungi

Organism	Temp℃	BOD (mg/L)	% Reduction	COD (mg/L)	% Reduction	O&G (mg/L)	% Reduction
Control	28	16280±25	-	78750±11	-	165 ±12	-
C. rugosa	25	8479±55	47	26306±65	66.6	155±31	6.1
	30	3140±28	80.7	25380±75	67.8	65±39	60.6
	35	2409±49	85.2	22170±76	71.8	54±25	67.3
	40	5427±33	66.7	27540±77	65	86±25	47.9
	45	7560±57	53.6	43480±98	44.8	115±36	30.3
	50	7650±27	53.0	47590±65	39.6	118±22	28.5
G. candidum	25	7354±55	54.8	33900±63	57	123±16	25.5
	30	3632±32	77.7	29651±75	62.3	73±15	55.8
	35	2070±30	87.3	28800±63	63.4	68 ±15	58.8
	40	6592±28	59.5	37560±78	52.3	96±25	41.8
	45	7459±45	54.2	35200±77	55.3	105±27	36.4
	50	7870±45	51.7	39199±72	50.2	135±18	18.2

**Values are the means of replicate determinations ± SD*

Table 4. Effect of soy bean concentration on biodegradation of POME using isolated fungi

Organism	Soy bean Conc. (w/v)	BOD (mg/L)	% Reduction	COD (mg/L)	% Reduction	O&G (mg/L)	% Reduction
Control	-	16280±25	-	78750±11	-	165 ±12	-
C. rugosa	1.5	10300±16	36.7	58300±47	26	145±13	12.1
	2.5	5300±12	67.4	46320±38	41.2	126±21	23.6
	3.5	2450±12	85	28000±35	64.5	77±14	53.3
	4.5	7704±16	52.7	65250±18	17.1	149±11	9.7
G. candidum	1.5	10200±51	37.3	68400±36	13.1	137±15	17
	2.5	3100±16	81	42300±18	46.2	64±13	61.2
	3.5	4650±19	71.4	59320±27	24.7	101±14	38.8
	4.5	8300±13	49	65030±25	17.4	124±11	24.9

**Values are the means of replicate determinations ± SD*

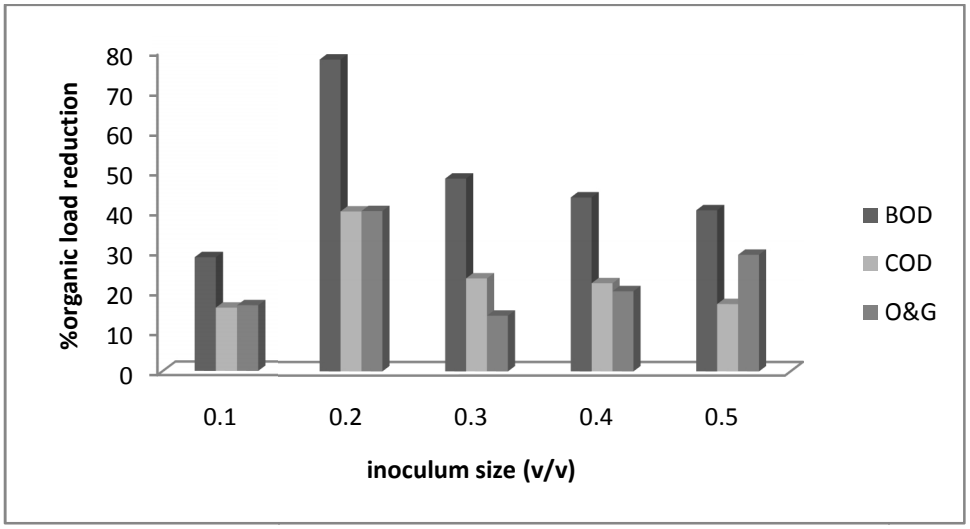

Fig. 2. Effect of inoculum size on biodegradation of POME using *G. candidum*

4. CONCLUSION

Palm oil mill effluents have existed for many years in southern parts of Nigeria but their effects on environment are currently being noticed due to urbanization. When POME is discharged untreated, they may cause serious problem and deteriorates the environment. This is due to the presence of high concentrations of BOD and COD in the effluents and hence it is preferable to recycle rather than discharging directly into the environment.

The present study using selected isolated fungi exhibited high efficiency of oil and grease removal as well as organic matter from POME. The isolates required control of environmental conditions and nutrient expansion for the effective biodegradation of POME.

Enhancement of environmental condition of indigenous microorganisms in biodegradation process requires control of pH, incubation temperature, inoculum size, agitation and substrate-related factors to bring up a flourishing achievement in aerobic waste degradation process. Aerobic degradation process used in this study saved time, cost and reduced of organic matter in POME.

Therefore the effect of aerobic treatment of POME on soil properties could help farmers in rural areas to improve in food production. This may be achieved by educating them on importance of treatment before application and required quantities depending on soil texture prior to planting.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahzad H, Sa'arib M, Mohamad AMS. Optimization of POME anaerobic pond. Europ. J. Sci. Res. 2009;32(4):455-459.
2. Ahmad AL, Ismail S, Bhatia. Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination. 2003;157(1-3):87-95.
3. Poh PE, Chong MF. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Biosour. Technol. 2008;1-9.
4. Cheng J, Zhu X, Ni J, Borthwick A. Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Biores. Technol. 2010;101:2729-2734.
5. Tubonimi TKI, Adiukwu PU, Stanley HO, Briggs AO. Impact of Palm oil (*Elaeis guineensis Jacq; Banga*) Mill Effluent on Water Quality of Receiving Oloya Lake, in Niger Delta, Nigeria. Research J. of Appl. Sci. 2007;2(7):842-845.
6. Lob KC, Tar PP. Effect of additional carbon sources on biodegradation of phenol. Bull Environ. Contamin. Toxicol. 2000;64:756–63.
7. Ma AN, Cheah SC, Chow MC. Current status of palm oil processing wastes management, Waste Management in Malaysia: Current Status and Prospects for Bioremediation. 2003;111-136.
8. Md Din, MF, Ujang Z, van Loosdrecht MCM, Ahmad A, Sairan MF. Optimization of nitrogen and phosphorus limitation for better biodegradable plastic production and organic removal using single fed-batch mixed cultures and renewable resources. Water Sci Technol. 2006;53:15–20.
9. Roux-Van Der Merwe MP, Badenhorst J, Britz TJ. Fungal treatment of an edible-oil-containing industrial effluent. World J. Microbiol & Biotechnol. 2005;21:947–953.
10. Husaini A, Roslan HA, Hii KSY, Ang CH. Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. World J. Microbiol. Biotechnol. 2008;24:2789–2797.
11. El-Bestawy E, El-Masry MH, El-Adl NE. The potentiality of free Gram-negative bacteria for removing oil and grease from contaminated industrial effluents. World Journal of Microbiol. Biotechnol. 2005;21:815–822.
12. Paparaskevas D, Christakopoulas P, Kekos, Macris, JB. Optimization production of extracellular lipase from *Rhodotorula glutinis*. Biotechnol. Lett. 1992;14:397–402.
13. Burkert JFM, Maugeri F, Rodrigues MI. Optimization of extracellular lipase production by Geotrichum sp. using factorial design. Bioresource Technology. 2004;91:77–84.
14. D'Annibale A, Sermanni GG, Federici F, Petruccioli M. Olive-oil wastewaters: A promising substrate for microbial lipase production. Bioresource Technology. 2006;97:1828–1833.
15. Grbavcic SZ, Dimitrijevic-Brankovic SI, Bezbradica DI, Siler-Marinkovic SS, Knezevic ZD.Effect of fermentation conditions on lipase production by *Candida utilis*. J. Serbian Chem. Soc. 2007;72(8–9):757–765.
16. Nwauche CO, Ogbonna JC. Isolation of lipase producing fungi from palm oil mill effluent dumpsite at Nsukka. Braz. Arch boil. Technol. 2011;54(1):113-116.
17. Ibegbulam-Njoku PN, Achi OK. Use of fungi in bioremediation of palm oil mill effluent (POME). Internat. J. Adv. Res. Technol. 2014;3(5):1-8. ISSN: 2278-7763.
18. APHA. Standard methods for the examination of water and wastewater. APHA AWWA-WEF, 20th Ed. Washington, DC; 1998.
19. American Standards for Testing and Materials, Standard Methods for Examination of Water and Wastewater, Washington D.C.; 2003.

20. Pechsuth M, Prasertsan P, Ukita M. Biopretreatment of palm oil mill effluent by thermotolerant polymer-producing fungi. (Suppl.) Oil Palm. Songklanakarin J. Sci. Technol. 2001;23:771-777.
21. Ngan MA, Tanisho S, Morimoto M, Yoshino S. Development of Conversion Technology of Biomass into Bioenergy. 2004;1-6. Reg no 2001EF002.
22. Wu TY, Mohammad AW, Jahim J, Md, Anuar N. Investigations on protease production by wild- type *Aspergillus terreus* using diluted retentate of pre-filtered palm oil mill effluent (POME) as substrate. Enzyme Microbial Technology. 2006;39(6):1223-1229.
23. Chaturvedi M, Singh M, Chugh MR, Kumar R. Isolation of Lipase Producing Bacteria from Oil Contaminated Soi*l.* Int. J. of Biotech and Biochem. 2010;6(4):585–594.
24. Agamuthu P, Tan EL, Shafal AA. Effect of aeration and soil inoculum on the composition of Palm Oil Mill effluent (POME). Agric. Waste. 1986;15:121 -132**.**
25. Gerardi MH. The Microbiology of Anaerobic Digesters. Wiley- Interscience, New Jersey. 2003;51-57.
26. Okwute LO, Isu NR. The environmental impact of palm oil mill effluent (POME) on some physico-chemical parameters and total aerobic bioload of soil at a dump site in Anyigba, Kogi State, Nigeria. African Journal of Agricultural Research 2007;2:656-662.
27. Gerardi, MH Wastewater Bacteria. Wiley-Interscience, New Jersey. 2006;19–31.
28. Parawira, W, Murto M, Zvauya R, Mattiasson B. Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate. Renew. Energ. 2006;31:893–903.
29. Patel H, Madamwar D. Effects of temperature and organic loading rates on biomethanation of acidic petrochemical wastewater using an anaerobic upflow fixed-film reactor. Biores. Technol. 2002;82:65–71.
30. Najafpour GD, Zinatizadeh AAL, Mohamed AR, Hasnain IM, Nasrollahzadeh H. High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochem. 2006;41:370–379.
31. Zekri AY, Chaalal O. Effect of Temperature on Biodegradation of Crude oil. Energy Sourc. 2005;27:1-2.
32. Khleifat KM. Biodegradation of phenol by *Ewingella americana*: Effect of carbon starvation and some growth conditions. Process Biochem. 2006;41:2010–2016.
33. Rekha KSS, Chandana LMVV, Sri Devi V, Siddartha KM. Production and optimization of lipase from *Candida rugosa* using groundnut oilcake under solid state Fermentation. Internat. J. Res. Eng. Technol. 2012;1(4):571–577.
34. Fadiloğlu S, Erkmen O, Effects of Carbon and Nitrogen Sources on Lipase Production by Candida rugosa. Turkish J. Eng. Env. Sci. 2002;26:249-254.
35. Bhumibhamon O, Koprasertsak A, Funthong S. Biotreatment of High Fat and Oil Wastewater by Lipase Producing Microorganisms. Kasetan J. Nat. Sci. 2002;36:261-267.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=605&id=8&aid=5633