Mapana J Sci, 15, 4 (2016), 23-34
ISSN 0975-3303| https://doi:10.12723/mjs.39.3

Enhancement of Biodegradation of Palm Oil Mill Effluents by local Isolated Fungi

F T Z Jabeen* and Shreevathsa J V†

Abstract

This study was designed to investigate the fungi associated with palm oil mill effluent (POME) in Gulur village of Tumkur. Biodegradation of palm oil mill effluents was conducted to measure the discarded POME based on physicochemical quality. The fungi that were isolated are *Aspergillusniger, A. flavus, A. fumigatus, A. ochraceus, Rhizopus*sp, *Peniciliium*sp and *Trichodermavirde.* The autoclaved and unautoclaved raw POME samples were incubated for 7 days and the activities of the fungi were observed each for 12 hours. The supernatants of the digested POME were investigated for the removal of chemical oxygen demand (COD), color (ADMI), and biochemical oxygen demand (BOD) at the end of each digestion cycle. The results showed that the unautoclaved raw POME sample degraded better than the inoculated POME sample and this suggests that the microorganisms that are indigenous in the POME are more effective than the introduced micro-organisms. This result, however, indicates the prospect of isolating indigenous microorganisms in the POME for effective biodegradation

* I. D. S. G. Govt. College, Chikkamagaluru 577102, Karnataka, India; ftzjabeen@gmail.com
† I. D. S. G. Govt. College, Chikkamagaluru 577102, Karnataka, India; srivathsasri710@gmail.com

Received: August 2016, Reviewed: September 2016

of POME. Moreover, the effective treatment of POME yields useful products such as reduction of BOD, COD, and color.

Keywords: Palm Oil Mill Effluent, Biodegradation, Gulur

1. Introduction

About 50 percent of the global traded vegetable oil comes from palm oil. The palm oil industry is a major agro-based enterprise especially in the southern part of the world where palm oil trees are found both in the wild and plantations [1]. Palm oil industries release POME in colossal amounts with its attendant pollution impending. POME has unfavorable environmental ramifications including land and aquatic ecosystem contamination and loss of biodiversity and increase in COD and BOD in environment [3]. The penetration of palm oil mill has been considered due to the ran off of its effluents into the waterways and ecosystems remaining a meticulous concern towards the food chain interference and water consumption [4]. This can cause considerable environmental problems if the effluents are discharged without effective treatment as they pollute land and effectively suffocate other aquatic life. Thus, palm oil mills are required to treat their POME prior to discharging it into rivers and streams. 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. 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).

The biological treatment depends enormously on consortium of microorganism's activities, which operate on the organic substances present in the POME as supplements and eventually degrade these organic matters into simple by-product such as methane, carbon dioxide and hydrogen supplied, and water. The biological treatment process requires large pond to hold the POME in place for the effective biodegradation, which regularly takes a few days relying upon the sort and native of the microorganisms [1]. Besides, so as to enhance the effectiveness of this medication process, powerful monoor combined cultures of feasible fungi in biodegradation treatment of POME waste are required. Therefore, the challenge of converting POME into an environmentally friendly waste demanded an efficient treatment and effective disposal technique.

This research intended to investigate the viability of fungi present in POME under specific conditions conducive for treatment. The process is investigated in terms of COD removal and decolorization (ADMI) with pH tolerance.

2. Materials and Method

2.1 Sample collection

Fresh palm oil mill effluent samples were collected from a local palm oil mill factory – Gulur village of Tumkur. Samples were collected using sterile screw cap 500 ml glass bottles, transferred immediately to the laboratory and were stored at 4^{O}C before further analysis. Raw POME has a dark brown color and pH of 4.2–4.5. The chemical characteristics of the POME are given in Table-1

2.2 Experimental Setup

Experiment was conducted in 250ml Erlenmeyer flasks that contained 100ml of POME that was covered with cotton gauze stopper. To study the effectiveness of microorganism's type on POME treatment, the batch tests were conducted under mesophilic condition. The medium was inoculated with inoculums culture (10% v/v) containing approximately 10^{6}cfu/ml of mixture of the isolated fungi in separate flasks and incubated at 37^{o}C on an incubator shaker (150rpm) for 7 days. The flasks containing the POME samples were autoclaved at 121^{o}C and 15 psi for 15 min. to produce the autoclaved samples. Both the autoclaved and raw flasks were inoculated with 5% (v/v) of the prepared inoculums. Three parameters were investigated in this study, such as the effects of BOD, COD, pH, and ADMI (Figures 1, 2, 3, and 4). The incubation period depends upon the microorganisms and inoculum size of the colony used. The culture was transferred to POME medium for preparation of seed culture.

Parameter	Concentration (mg/l)
Biochemical oxygen demand	(BOD) 23,400-52,100
Chemical oxygen demand	(COD) 80,100-95,000
Total carbohydrate	17,000-19,000
Total nitrogen	850-930
Ammonium nitrogen	24-31
Total phosphorus	96-120
Phosphate	15.2-20.6
Oil	8,500-11,000
Total solid	36,000-43,000
Suspended solid	9,400-12,500
Ash	4,200-4,6000

Table 1: Chemical characteristics of palm oil mill effluent (POME) used in this study

2.3 Identification of Fungi

Identification of fungi was based on the macroscopic and microscopic morphology [5]. The observations with microscopic and macroscopic characteristics were compared, respectively. The fungi isolated from the POME included *Aspergillusniger, A. flavus, A. fumigatus, A. ochraceus, Rhizopus*sp., *Peniciliium*sp., *Fusarium*sp., and *Trichodermavirde* inoculated into the autoclaved sample and then inoculated sample, based on isolated microorganisms was incubated (10^6cfu/ml) at 37^{o}C and agitated at 150 rpm.

2.4 Analytical Methods

To separate the fungal biomass and the liquid medium, the whole fungi culture was centrifuged at 4,000 rpm for 15 min at 4^{o}C. Analytical parameters of POME were analysed based on the methods developed and modified by the Department of Environment [6], such as COD (digestion method), BOD (biochemical oxygen demand), and the color (ADMI unit), which were determined according to the APHA method [7]. The pH was measured using pH meter. All experiments were conducted in triplicates.

2.5 Determination of COD Removal

The analysis of COD was carried out [8] by using 1 ml of the supernatant of each treated sample was transferred into COD vial and it was diluted with 0.9ml distilled water (COD 0–1500 ppm range) and the mixture was mixed gently. The vial was placed in the preheated COD reactor at 150°C for 2 hours. The percentage of COD removal was determined by cooling it at room temperature for 1 hour and determined by the spectrophotometry method. The absorbance was read at 620 nm (COD 0–1500 ppm range using HACH program). For the blank, deionised water was used for the background subtraction. All analyses were run in triplicates [9].

2.6 Determination of Biochemical Oxygen Demand

BOD test is a widely used parameter for evaluating the ability of naturally occurring microorganisms to digest organic matter, This experiment was done by dilution method [10]. A measured amount of the biodegradation sample was poured into the 300ml BOD standard bottle. In 3–7 days incubation at 37∘C by analyzing the depletion of oxygen inside the POME.

2.7 ADMI (American Dye Manufacturers' Institute Colour)

The color was determined by recording spectrophotometer [11]. The supernatant was also used to determine the decolorization of POME. Thus, 0.1ml of the supernatant was diluted with 3.9ml of phosphate buffer. They were mixed homogenously prior to transfer into the cuvette to be quantified of their degree of decolorization. The quantification was done by measuring the reduction of optical density (OD) at 471 nm.

3. Results and Discussion

A total of 8 fungal colonies were isolated from POME and cultured on Potato dextrose agar medium. Identified *Aspergillussp.* are associated with lipase production. Lipase accelerates in hydrolysis of lipid causing consequent breakdown into fatty acid and alcohol [12]. *Aspergillusniger* and *Aspergillusflavus* have been distinguished for their ability to endure oily waste water such as POME. The presence of *Penicillium*sp., *Fusarium*sp., and *Rhizopus*sp. in the POME shows that these fungi are able to survive in hostile environment [13,14]. According to previous reports, these organisms can degrade oily waste water effectively.

The value of pH recorded in through this study is lower, that is, more acidic than the Ohimainet al. [15]. The low pH of the POME indicates that it is acidic and should be treated to reach 7–7.5 pH as indication level of plant compatible. The acidic character of POME may have been influenced by the corrosion of iron used in processing. When the POME is discharged into the soil or stream, it affects nutrient availability of the nearby plants [16]; in addition, most plants grow within a pH range of 6.5–7.5 [17].

Decolorization is one of the vital parameters in POME quality assessment and indicates the physical and biological processes prevailing in the POME; it points to the degree of pollution in wastewater [18]. The predominate microorganisms in POME have been variously mobilized for the treatment of the wastewater [19], [20]. During degradation, oil and grease in the POME are broken down and mineralized. However, the microbial content of POME is a good indicator of biodegradability of wastewater. However, since most of these organisms are spore formers, it helps them to survive the harsh environmental conditions of POME such as acidity [21], [22]. Moreover, the dark brown color of palm oil mill effluent is composed of organic compounds such as anthocyanin and carotene pigment that were extracted from fresh fruit bunches in the sterilization process[23]. Furthermore, it included polyphenol compounds, tannin, polyalcohol, and melanoidin [23, 24].

3.1 COD Concentrations of Digested Samples

The COD removal for the biodegradation of POME samples is depicted in Figure 1. In general there was gradual decrease in the COD concentrations of the raw POME sample including the inoculated organism which was selected in this study and control. Furthermore, it was observed that the removal of COD in the auto clave sample was very close to the raw sample and this may suggest inoculated sample with isolated microorganism in the raw sample. In addition, the activities of local microorganisms may be used to describe the degradation of raw POME with high degradation of POME.

3.2 Change in pH of Digested Samples

The pH of the entire biodegradation samples increased from acidity towards alkalinity; processes increase alkalinity at different rate and dimensions (Figure 2). The control raw (RAW CNTL) sample and those inoculated with selected microorganisms respectively, reached neutral point at the end of the period and one important point to note is that treatment should reach high standard of pH of POME for release through the environment. A pH of 7.6 could vastly increase the benefits, since the treatment of the POME is for plant reutilization and discharge into river streams with less effect on the environment. Furthermore, the pH of the autoclaved samples is the case in the study which was normally below the pH of the un-autoclaved samples except for sample with microorganism treatment.

3.3 Changes in Biochemical Oxygen Demand (BOD) of Samples

The changes in the biochemical oxygen demand (BOD) of the samples were checked out for seven days (164 hours) in the BOD incubator (Figure 3). The concentrations of the BOD generally decreased with number of days for all the samples inoculated with multi micro organisms as well as the control sample but treatment shows stability in final stage. This trend is positive to the biodegradation treatment process of high strength wastewater such as POME, because the decrease in the BOD indicates the reduction in the environmental organic load on the receiving water stream.

3.4 Changes in ADMI of Samples

The changes in the color of the samples were checked out for seven days (164 hours) based on the ADMI method at 471 nm (Figure 4). The concentrations of the color generally decreased with the number of days for all the samples inoculated with fungi as well as the control sample but treatment shows stability in final stage, whereas there are fluctuation for control group which indicates contradiction in metabolites of microorganism system (growth between 60–71 hours and 132 hours). This treatment shows positive tendency in biodegradation treatment of high strength waste water such as POME, because the decrease in the color indicates the reduction in the environmental organic as a control application.

3.5 Identification of the Efficient COD and BOD Removal

The COD and BOD reduction performance was monitored each 12 hours in batch system consisting of fungi isolated from POME wastewater. The adaptation of the mesophilic microorganism operating condition at 37°C led to a stable process that is significantly different from that previously found at 37°C (Table 2).This new process allows for a conversion of organic matter into the final treatment. Among eight fungi identified in POME six fungal species were persistent. The persistent fungi were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Fusarium, Penicillium,* and *Rhizopus*. High population of fungi in the POME may be associated with contaminations from poor sanitation in the mills. The microbial species found in POME has the potential of degrading hydrocarbon in the wastewater.

Biodegradation is associated with the saprophytic ability of fungi to grow on and degrade carbon sources in industrial effluents and reduce factor which could influence the environment. In this study, the acidic tendency of POME treatment (Table 2) may affect nutrient availability of the nearby plants which pasture from discharge of POME in river; final pH reached approximately 7.6 (Ut RAW) in appropriate range rather than RAW-CNT and AUTO-CNTL (6.5 and 5.4, resp.) (Figure 2); most plants grow and do better within a pH range of 6.5–7.5. BOD is an important parameter in POME quality measurement and indicates the physical and biological processes surpass in the POME biodegradation; it indicates the degree of pollution in wastewater, and BOD value obtained during the study was in the range of 155000–168000mg/L (Figure 3). COD was an important aspect to estimate the organic content in POME. The effect of different oxygen concentration on the reduction of COD was depicted in Figure 1. It implies that the population of microorganisms plays a vital role in the process of reduction of COD, thus *purged* wastewater. Figure 1 showed that the number of fungi increases and at the same time causes the reduction in COD concentration of POME, approximately 12000 mg/l in 168 hours of treatment. Figure 4 presents the colorization which shows positive tendency in biodegradation treatment of high strength POME; color measurement indicates the reduction in the environmental organic 486 to 180 ADMI.

3.6 Optimum Physicochemical Conditions for Growth

The growths of the potential isolated fungi were determined at different temperatures. Substantial growth was observed at temperatures 35–37^{o}C. In the case of growth at different condition with mixture of local microorganism, the optimum temperature had maximum growth at 37^{o}C and agitated at 150 rpm.

4. Conclusion

Environmental issues have been gradually increased and becoming one of the most important in economic activities. In addition, due to the high concentrations of BOD and COD in the oil palm processing effluents, discharging it into the environment could cause pollution; hence, it is preferable to recycle rather than discharging it. By improving environmental condition of living microorganisms in a biodegradation process, substrate-related factors could be utilized in bringing up a flourishing achievement in waste degradation process. The addition of functional inoculum and selected high effective indigenous microorganisms is expected to save the POME degradation time and cost for reduction of BOD, COD, and color.

Parameter	Physichemical Properties of POME		
	Current (mean ± standard error)	Ohimain *et al.*, 2012	Ohimain *et al.*, 2012
pH	4.63-4.70	5.123-6.375	6.56±0.05
ADMI	2.355-3.766	2.567-4.127	4.69±0.00
COD, mg/l	85340-11835	1806.33±7.12	1806.33±7.12
BOD, mg/l	48555-48994	254-1541	382.93±0.89

Table 1: Physicochemical properties of palm oil mill effluent (POME) used in this study

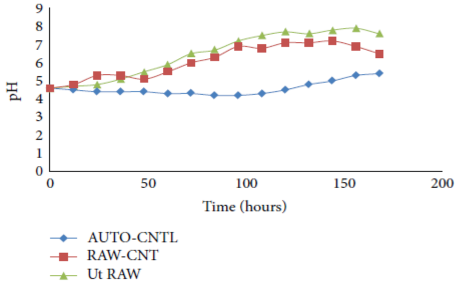

Figure 1 BOD (mg/l) of autoclaved (AUTO-CONTL) and unautoclaved (RAW-CONTL) samples digested with inoculated fungi (Ut RAW). The control sample was not inoculated (RAW-CONTL)

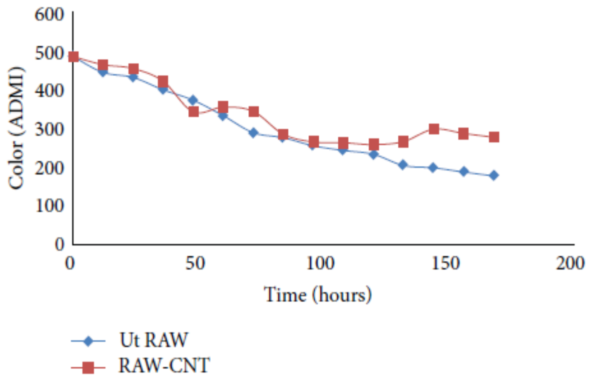

Figure 4 Colour (ADMI) of autoclaved (AUTO-CONTL) and unautoclaved (RAW-CONTL) samples digested with inoculated fungi (Ut RAW). The control sample was not inoculated (RAW-CONTL)

References

[1] V. O. Nwaugo, G. C. Chinyere and C. U. Inyang. Effects of Palm Oil Mill Effluents (POME) on Soil Bacterial Flora and Enzyme Activities in Egbama. Plant Prod. Res. J.vol.12, pp. 10-13, 2008.
[2] T. Y. Wu, A. W. Mohammad, J. M. Jahim, and N. Anuar. Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. Journal of Environmental Management.vol. 91, pp. 1467–1490, 2010.
[3] P. Singh nee'Nigam and A. Pandey. Biotechnology for Agro-Industrial-Residues-Utilisation. vol. 1, Springer Science+Business Media B.V., New York, NY, USA, 2009.
[4] J. Cheng, X. Zhu, J. Ni, andA. Borthwick. Palmoil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresource Technology.vol. 101, pp. 2729–2734, 2010.
[5] M. Ayyachamy, K. M. Turner, A. O'Donovan, V. K. Gupta, and M. G. Tuohy, Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology, Springer, New York, NY, USA, 2012.
[6] Malaysia, D. o. E. o., Revised Standard Methods (1985) for Analysis of Rubber and Palm Oil Effluents, 2nd edition, 1995.
[7] A. D. Eaton and M. A. H. Franson. Standard methods for the examination of water & wastewater, 2005.
[8] A. M. Jirka and M. J. Carter. Micro semi-automated analysis of surface and wastewaters for chemical oxygen demand. Analytical Chemistry.vol. 47, pp. 1397–1402, 1975.
[9] A. P. H. Association and A. W. W. Association. Standard methods for the examination of water and wastewater. 1976.
[10] K. Riedel, R. Renneberg, M. Kuhn, and F. Scheller. A fast estimation of biochemical oxygen demand using microbial sensors. Applied Microbiology and Biotechnology, vol. 28, pp. 316–318, 1988.
[11] N. Abdullah, Z. Ujang, and A. Yahya. Aerobic granular sludge formation for high strength agro-based wastewater treatment. Bioresource Technology.vol. 102, pp. 6778–6781, 2011.
[12] P. Ghosh, R. Saxena, R. Gupta, R. Yadav, and S. Davidson. Microbial lipases: production and applications. Science Progress.vol. 79, pp. 119–158, 1996.
[13] E. I. Ohimain, C.Daokoru-Olukole, S. C. Izah, R. A. Eke, and A.C. Okonkwo. Microbiology of palm oil mill effluents. Journal of Microbiology & Biotechnology Research, vol. 2, pp. 852,2012.

[14] E. I. Ohimain, E. I. Seiyaboh, S. C. Izah, V. Oghenegueke, and T.Perewarebo. Some selected physico-chemical and heavy metal properties of palmoil mill effluents. Greener Journal of Physical Sciences.vol. 2, pp. 131–137, 2012.
[15] E. I. Ohimain, S. C. Izah, and N. Jenakumo. Physicochemical and microbial screening of palm oil mill effluents for amylase production. Greener Journal of Biological Sciences.vol. 3, pp. 307–318, 2013.
[16] L.O. Okwuteand N. R. Isu. 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, vol. 2,pp. 656–662, 2007.
[17] B. Hajek, D. Karlen, B. Lowery et al., Erosion and soil properties in Proceedings of Soil Erosion and Productivity Workshop, University of Minnesota, St. Paul, Minn, USA, 1990.
[18] H. M. Gopalkrushna. Assessment of physico-chemical status of ground water samples in Akot city. Research Journal of Chemical Sciences. vol. 1, pp. 117–124, 2011.
[19] S. A. Kamal, J. M. Jahim, N. Anuaret al., Pre-treatment effect of palm oil mill effluent (POME) during hydrogen production by a local isolate clostridium butyricum. International Journal on Advanced Science, Engineering and Information Technology.vol. 2, pp. 54–60, 2011.
[20] M. N. Ahmad, M. N. Mokhtar, A. S. Baharuddinet al., Changes in physicochemical and microbial community during co-composting of oil palm frond with palm oil mill effluent anaerobic sludge. BioResources.vol. 6, pp. 4762–4780,2011b.
[21] E. O. Ugoji. Anaerobic digestion of palm oil mill effluent and its utilization as fertilizer for environmental protection. Renewable Energy.vol. 10, pp. 291–294, 1997.
[22] C. P. Leslie Grady Jr., G. T. Daigger, and H. C. Lim. Biological Wastewater Treatment, CRC Press, Montreal, Canada, 1999.
[23] R. R. Mohammed, M. R. Ketabachi, and G. McKay. Combined magnetic field and adsorption process for treatment of biologically treated palm oil mill effluent (POME).Chemical Engineering Journal.vol. 243, pp. 31–42, 2014.
[24] M. B. Agustin, W. P. Sengpracha, and W. Phutdhawong. Electrocoagulation of palm oil mill effluent. International Journal of Environmental Research and Public Health.vol. 5, pp.177–180, 2008.