Pak. J. Biotechnol. Vol. 14 (2) 221- 225 (2017)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312- 7791

POTENTIAL OF TREATING UNSTERILIZED PALM OIL MILL EFFLUENT (POME) USING FRESHWATER MICROALGAE

Saidu Haruna[1,3], Shaza Eva Mohamad[2*], Haryati Jamaluddin[1],

1. Faculty of Bioscience and Medical Engineering, University Teknologi Malaysia, 81310, Johor Bahru, Malaysia. 2. Malaysia Japan International Institute of Technology, Universiti Teknologi Malaysia Kuala Lumpur 54100 Kuala Lumpur, Malaysia. 3. Department of Biological Sciences, Faculty of Sciences, Gombe State University, 127, Gombe State, Nigeria. E-mail: shaza@utm.my

Article received 13.3.2017, Revised 26.4.2017, Accepted 3.5.2017

ABSTRACT

Untreated Palm Oil Mill Effluent (POME) is a wastewater that contains high amount of chemical substances that when discharge directly into the waterways causes acute water pollution crises especially to Malaysia where palm oil is cultivated extensively. This research was carried out in order to evaluate the potential of utilizing *Chlorella sorokiniana* for the phycoremediation of POME. *C. sorokiniana* grew in POME under different dilutions of 80%, 60%, 40% and 20%. *C. sorokiniana* removed nitrate, total phosphorus, chemical oxygen demand (COD) and ammonium at ranges of 33-71%, 29-64%, 19-91% and 70-98% over 15 days culture. This study proved the successful cultivation of microalgae in unsterilized POME which could contribute to the effort in finding effective method of treating POME

Key words; Palm oil mill effluent, water pollution, phycoremediation, microalgae

INTRODUCTION

Palm Oil Mill Effluent is a wastewater generated from palm oil industry containing large amount of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and heavy metals. Direct discharge of this effluent into the water course causes severe pollution to the receiving water body which is the reason why Malaysia is currently experiencing water pollution crises (Abdullah et al. 2011; Saravanan et al., 2017). Furthermore, the situation where we are currently faced with more stringent regulations, wastewater pollutants are the environmental priority pollutants and are becoming one of the most serious environmental problems (Saidu et al., 2017). So, these toxic chemicals should be removed from the wastewater to protect the people and the environment.

The application of microalgae for the bioremediation of wastewater has been implemented since and was reported to be successful (Wang et al., 2016; Hasan et al., 2016). Recently, Ding and his co-workers (2016) cultivated *Chlamydomonas* sp under different dilutions of POME. They reported the removal of COD, TN, $NH4^+$-N and TP at range of 8.59%-29.13%, 43.5-72.97%, 58.58%-100% and 38.15%-63.53% respectively. The combine culture of microalgae and bacteria for wastewater treatment has been shown to yield efficient treatment results (Muñoz and Guieysse 2006). Su et al., (2011), has shown that Microalgae-bacterial culture has the efficiency of nitrogen and phosphorus removal of 91.0±7.0% and 93.5±2.5% in 14 days when cultivated in wastewater. Furthermore, Mujtaba and his co-workers (2015) cultivated *Chlorella vulgaris* and *Pseudomonas putida* in synthetic municipal wastewater. The result showed that the co-culture system removed ammonium and COD around 80%.

Therefore, most of these researches mainly focus on the application of microalgae or microalgae-bacteria culture for the treatment of wastewater, the novelty of this study lies on the cultivation of mixotrophic microalgae (*C. sorokiniana*) with the bacterial community for the treatment of POME. The aim of this study is to investigate the effectiveness of utilizing the symbiotic culture of freshwater microalgae, *Chlorella sorokiniana* and bacterial community for the pollutants removal in POME under different POME dilutions.

MATERIALS AND METHODS

Sample collection: POME was collected at the facultative anaerobic pond (FAP) from local palm oil mill industry (Kilang Sawit Bukit Besar, Johor Bahru Malaysia). POME was left to settle for 4 hours before filtered using a muslin cloth and whatman's filter paper. The reason of filtration is to remove free suspended materials. For the purpose of preservation, POME was refrigerated at 4°C to prevent biodegradation (Ding et al., 2016).

Algae and Culture condition: Microalgae (*C. sorokiniana*) was cultivated and maintained on Proteose media at 29°C. The composition of Proteose media consist of $NaNO_3$ (25 g/L), $CaCl_2.2H_2O$ (2.5 g/L), $MgSO_4.7H_2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L), Urea (1.5 g/L). pH of the media was kept at 7 using NaOH. 10% (v/v) inoculations of algae were carried out in 1 L flask containing 500 mL media. The cultivation was done under constant aeration with two fluorescent lamps made up 3000 lux providing a source of illumination for the 12h day: night interval.

Cultivation of *C. sorokiniana* in unsterilized POME: The cultivation of microalgae was done following method of Wang et al. (2010) but with modification. 20% (v/v) of well-grown microalgae was cultivated in unsterilized POME under different dilutions of 80, 60, 40 and 20% (v/v). Different dilution of POME to distilled water was prepared and about 10% inoculums of *C. sorokiniana* was cultured photo-autotrophically. Under the above-mentioned condition, four dilutions, 80%, 60%, 40%, and 20%, of POME was made in distilled water. The cultivation was maintained under a continuous supply of air, 12 h light, pH 7, and the temperature (25-30ºC) in 1 Litre Erlenmeyer flask. The justification of using *C. sorokiniana* for this experiment is because of the following reason; (1) Ability to grow under mixotrophic mode. (2) Robust feature which enable it to withstand high toxic level of wastewater.

Characterization of POME: The characteristics of POME collected are shown in Table **1**. Nitrate, phosphate, and ammonium were determined following the Hatch DR 6000^{TM} Spectrophotometric Manual (DR/6000, Hatch Co. Ltd. Tokyo 2008)**.** Nitrate and phosphate were specifically determined using cadmium reduction method and an acid hydrolysable method respectively while ammonium was determined by Nessler method (Wang et al., 2010). Suitable dilutions were made for the high concentration and the final results were computed by multiplying the dilution factor. pH was determined using the portable pH meter, total suspended solid (TSS) was determined according to the standard methods (APHA, 2005). To determine the concentration of heavy metals in POME, 10 mL of POME was acid digested using 2.5 mL concentrated nitric acid (65% HNO_3) and 0.8 mL of hydrochloric acid (HCL) at 180ºC for 20 min in a microwave oven (model; Berghof *Speed wave 4*). Afterward, the digested samples were taken out, allowed to cool, filtered and diluted with distilled water. The sample was then analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) according to the method portrayed by (Meche et al., 2010). All the data were analyzed using Microsoft excel 2010. The percentage removals of nitrate, phosphate, and ammonium were computed using the formula;

$$\% = \frac{Ci}{Cf} \times 100$$

where Ci = Initial concentration and Cf = is the final concentration.

RESULTS AND DISCUSSION

The characteristic of anaerobic digested POME was measured and summarized in Table 1. The initial results of characterization showed that, POME contain COD, nitrate, total phosphate and ammonium of 2100 mg/L, 181 mg/L, 131 mg/L and 245 mg/L respectively. Interestingly, this indicated that, POME contain high nutrients load which could be used as a medium for the cultivation of microalgae. The reason for selecting these four parameters for their removal using *C. sorokiniana* is because they formed the major sources of nutrients necessary for the cultivation of microalgae. Compare with the standard discharge limit, the values of these parameters were above the permissible discharge limit. Therefore, combine treatment using algae and bacteria will beneficial in ensuring that, these nutrients are largely removed before its discharge out (Rupani et al. 2010).

Table 1: General characteristic of POME from anaerobic pond

General Characteristics	POME[a]	Malaysian discharge limit[b]
pH	6.5	5-9
COD	2100	-
TSS	3200	400
Nitrate	181	-
Phosphate	131	-
Ammonium	245	150
Turbidity	420	

[a] All parameters unit is in mg/L except pH and turbidity (NTU). [b] Reference; (Rupani et al., 2010)

The nutrients consumption profile of *C sorokiniana* in unsterilized POME is shown in Figure 1-4. There was a remarkable COD reduction in all the wastewater dilutions. Wastewater consists of organic compounds containing at least one carbon atom, which is oxidized chemically to liberate carbon dioxide. COD indirectly measures the amount of organic and inorganic compounds present in wastewater. High amount of COD in wastewater pollute water by causing dead of aquatic biota. However, Hadiyanto et al., (2013) repor-ted that, microalgae are capable of converting COD into carbon sources and utilized for photosynthesis.

The highest COD removal of 90% was observed in 80% dilution, followed by 86%, 27%, and 19% COD removal in 60, 40 and 20% dilutions of POME respectively. The active utilization of COD removal indicated that microalgae and bacteria are involved in the COD degradation in POME. The COD removal in 80 and 60% dilution were almost at the same parallel line trend between days 9 to 15, causing *C. sorokiniana* to reach stationary phase which indicated that, COD as a source of carbon is limiting to the microalgae growth in 80 and 60% dilution (Fig. 1). COD removal in low diluted unsterilized POME was found to be stagnant, which is due to volatile organic carbon removal during bubbling (aeration) (Ji et al., 2015). There was no significant COD removal by algal under 20% dilutions between days 6 to 12 as the microalgae is already at the stationary phase, following the next 3 days, the COD content showed a significant removal. In the case of 40% dilution, COD removal was found to be stagnant between days 9 to 15 culture.

The percentage of COD removal was found to be efficient in diluted POME than the concentrated POME media. This is most likely due to *C. sorokiniana* growing in the medium with sufficient light penetration thereby adopting phototrophic mode of growth. In the case of the concentrated POME media, although microalgae adopt mixotrophic mode of growth; the low light penetration can be one of the priority factor that can affect the growth of algae.

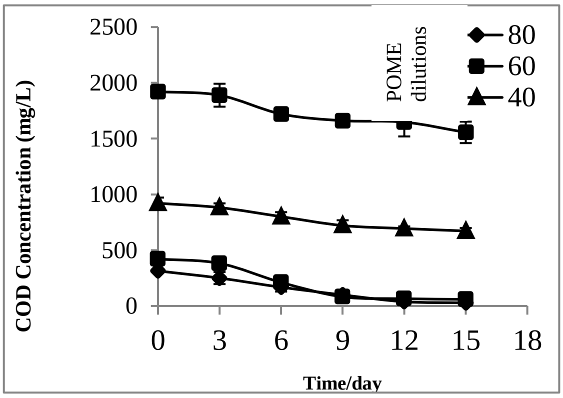

Fig. 1. COD consumption in the medium of different dilutions of unsterilized POME with *C. sorokiniana* growth

Nitrogen is one the primary element necessary for microalgae growth and metabolism. The various forms of nitrogen available in POME are nitrate, nitrite, and ammonium. The percentage removal of nitrogen by *C. sorokiniana* in 80, 60, 40, 20% dilution was found to be 71, 50, 41, and 33% respectively. The nitrogen removal curve in Figure 2 showed that the highest nitrogen removal was found to be under 71% dilution while the least was 20% dilution. Ammonium was removed greatly in 80% dilution, followed by 60, 40, and 20% dilution respectively. The percentage removal of ammonium in the four POME dilutions was found to be 98%, 93%, 72%, and 70% respectively. The maximum ammonium utilization of 98% compare to 80% nitrate utilization, suggested that microalgae prefer ammonium as a best nitrogen source in POME. This was also reported by Wang et al. (2010) when treating digested dairy manure effluent.

The nitrate removal curve was shown to have parallel trend line with that of ammonium except in 80 and 60% dilution (Fig. 2 and 3). This trend of nitrate and ammonium removal was found to vary with the research of Ding et al. (2016) in which the removal curves of both nitrate and ammonium consumption using *Chlamydomonas* sp. in sterile POME was found to be parallel in all the dilutions. This could be due to the differences of microalgae species used for the treatment, culture condition (sterilized or unsterilized) and the size of the dilution factor. Furthermore, it indicated that only inorganic nitrogen such as ammonium and nitrate can be utilized by microalgae. Even though Ding et al. (2016) reported 100% NH_4-N removal by *Chlamydomonas* sp, in this study, the total NH_4-N removal in unsterilized POME was found to be 98%, suggesting that, the presence of bacteria in the culture system does not provide much effect. In addition, *C. sorokiniana* may have attained the level of saturation with ammonium in wastewater than *Chlamydomonas* spp.

Furthermore, the ammonium consumption trend of *C. sorokiniana* in 80 and 60% dilutions were found to be parallel between days 9-15 unlike in the case of nitrate consumption curve where the parallel nitrate removal occurs between only between days 9-12.

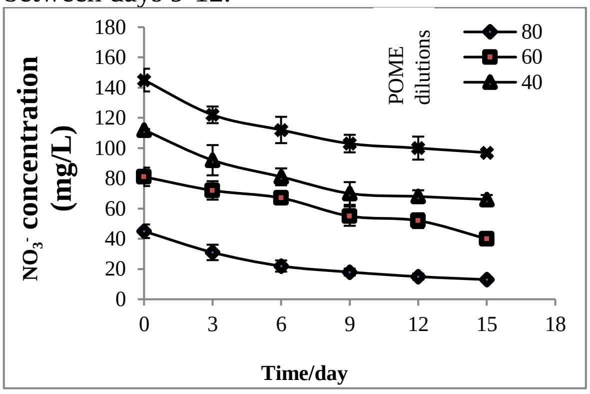

Fig. 2. Nitrate consumption in the medium of different dilutions of unsterilized POME with *C. sorokiniana* growth

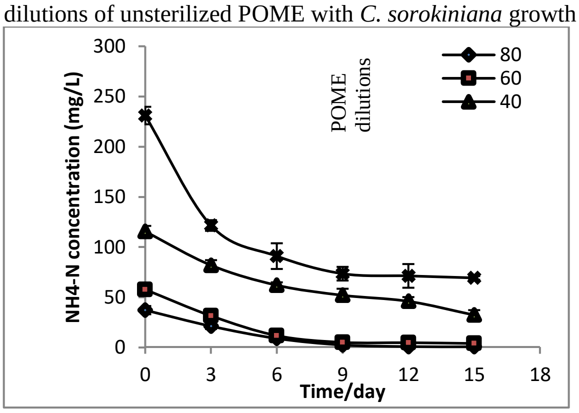

Fig. 3. Ammonium consumption in the medium of different dilutions of unsterilized POME with *C. sorokiniana* growth

Phosphorus can also be classified as another necessary element requires by microalgae for growth and energy generation. Based on the phosphorrus removal efficiency, *C. sorokiniana* removed phosphate of about 64%, 44%, 40% and 29% in 80%, 60%, 40% and 20% dilutions respectively. This indicated that *C. sorokiniana* showed high activities for total phosphorus removal in 80% dilution than 60, 40 and 20% dilution POME. The percentage rate of phosphorus removal reported from this study was slightly lower than 85.92% phosphorus removal by *Spirulina platensis* in POME (Hadiyanto et al., 2013). The phosphorus removal curve in Figure 4 showed that phosphorus content in all the dilutions was still high, which is an indication that, phosphorus was not the limiting factor for growth. A parallel trend line of the total phosphorus removal was observed under 80% and 60% dilutions unlike 20% and 40% dilution which depict some irregularity in the pattern of phosphorus consumption (Fig. 4). Microalgae absorbed phosphorus and transformed it into Adenosine Tri Phosphate (ATP). Microalgae utilized ATP as a source of energy for photosynthesis and metabolism (Hadiyanto et al., 2013). However, high concentration of phosphorus in the medium affect microalgae growth by causing toxicity to the growth medium which is the reason why *C. sorokiniana* could not remove phosphorus at high amount under 20% and 40% dilution. However, microalgae showed remarkable TP removal in all the dilutions.

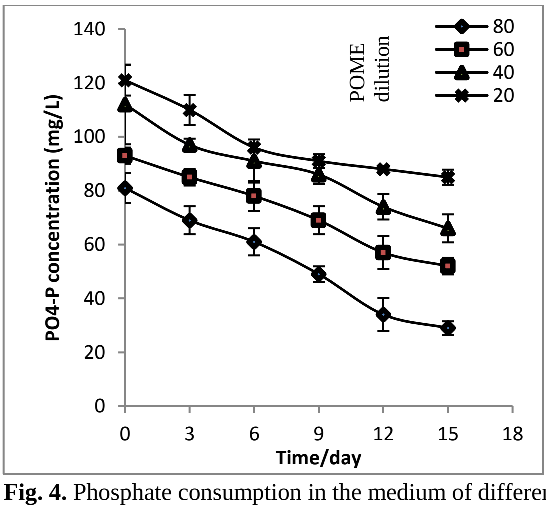

Fig. 4. Phosphate consumption in the medium of different dilutions of unsterilized POME with *C. sorokiniana* growth

The culture of *C. sorokianna* in unsterilized POME have shown that this system performed greatly by ensuring efficient nitrate, phosphate, ammonium, and COD removal all the dilutions. It was noted that nutrients removal by *C. sorokiniana* was found to be better under 80% dilution than 60, 40, and 20%. This is because, the nutrients concentration of 60, 40, and 20% dilutions is high and this might cause toxicity to the growth of algae as reported by (Chinnasami et al., 2010). Also, light penetration constitutes important parameters for growth of microalgae (Mangi et al., 2017). 80% dilution received the highest dilution with distilled water, as such; light penetration will be intense in that dilution. This suggested that, that nutrients removal by *C. sorokiniana* in 80% was due to autotrophic growth, whereas in the remaining dilutions, there is insufficient light penetration, thus the growth of algae in those dilutions was due to mixotrophic growth. The experimental picture can be seen in Fig. 5. Because the POME, used for the cultivation of microalgae was anaerobically digested, the acetogenic bacteria convert the volatile fatty acid to acetate, and methanogen bacteria convert the produced acetate into the methane and carbon dioxide. Thus, the resulting acetate for microalgae growth was due to the partial breaking down of acetate to methane by methanogen bacteria. The findings of this research are very significant in contributing to the effort of overcoming the shading effect of POME which affects microbial growth. Furthermore, the study proposed a suitable methodology of culturing microalgae for enhance growth and efficient nutrient removal.

Fig. 5. Experimental pictures of algae in POME

CONCLUSION

The potential of cultivation of algae and bacteria for the treatment of POME was investigated in this study. Based on the overall results of the study, *C. sorokiniana* grew well in unsterilized POME. *C. sorokiniana* under unsterilized condition removed nitrate, phosphate, COD and ammonium at a range of 33-71%, 29-64%, 19-91%, and 70-98% respecttively. The results of the study concluded that 80% dilution of POME can be used as suitable medium for growth and nutrients removal of the co-culture of *C. sorokiniana* with bacteria. The nutrients removing ability of *C. sorokiniana* was found to be inhabited by the shading effect especially in high concentrated POME. Result of this study is significant as it will contribute in the effort of finding an efficient method of culturing algae for efficient bioremediation of POME

Acknowledgement

The Author wishes to acknowledge the financial support received from Universiti Teknologi Malaysia for the funding of this research under grant 08h27.

REFERENCES

Abdullah. N., Z. Ujang, and A. Yahya, Aerobic granular sludge formation for high strength agro-based wastewater treatment. Bioresour. Technol. 102: 6778-81 (2011).

Chinnasamy, A., R.W. Bhatnagar, K.C. Hunt, K.C. and Das, Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour. Technol. 101: 3097-3105 (2010).

Ding, G.T., Z. Yaakob, M.S. Takriff, J. Salihon, and M.S.A. Rahaman, Biomass production and nutrients removal by a newly-isolated microalgal strain *Chlamydomonas* sp in palm oil mill effluent (POME). Int.J.Hydrogen Energy 41(8): 4888-4895 (2016).

Federation, W.E. and A.P.H. Association, Standard methods for the examination of water and waste -water. American Public Health Association (APHA): Washington, DC, USA (2005).

Hadiyanto, M.C. and Soetrisnanto, D., Phytoremediations of Palm Oil Mill Effluent (POME) by using aquatic plants and microalge for biomass production. J. Environ. Sci. Technol. 6(2): 79-90 (2013).

Hasan, S.A. A., Aziz, K. Hassan, S. I. Ali, and S. Jabeen, Production of Biodiesel From Freshwater Algae. Pak. J. Biotechnol. 13 (4): 267 – 274 (2016).

Ji, M.K., R.A. Abou-Shanab, S.H. Kim, E.S. Salama, S.H. Lee, A.N. Kabra, and B.H. Jeon, Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO 2 for nutrient removal and biomass production. Ecological Engineering 58: 142-148 (2013)

Lam, M.K., and K.T. Lee, Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection. Biotechnol. Adv. 29: 124-141 (2011).

Mangi, J., N. Soomro, S.A. Tunio, A.H. Memon, M.A. Kaz, and A.R. Jamali, Phycochemical studies on microspora floccosa (vaucher), thuret alga collected from the ponds of hyderabad, sindh. Pak. J. Biotechnol. 14 (1): 83-86 (2017).

Meche, A., M.C. Martins, B.E. Lofrano, C.J. Hardaway, M. Merchant, and L. Verdade, Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil. Microchem. J. 94: 171-74 (2010).

Mujtaba, G., M. Rizwan, and K. Lee, Simultaneous removal of inorganic nutrients and organic carbon by symbiotic co-culture of *Chlorella vulgaris* and *Pseudomonas putida*. Biotechnol. and Bioprocess Eng. 20(6): 1114-1122 (2015).

Munoz, R. and B. Guieysse, Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water research. 40(15): 2799-2815 (2006).

Rupani, P.F., R.P. Singh, M.H. Ibrahim, and N. Esa, Review of current palm oil mill effluent (POME) treatment methods: vermicomposting as a sustainable practice. World Appl. Sci. J. 11(1); 70-81 (2010).

Saidu, H., Jamaluddin, H., Iwamoto, K., Yahya, A., Mohamad, S.V. Low-cost biodiesel production. Asian Journal of Applied Science 10(2): 57-65 (2017(.

Saravanan, D. and S.V. Lakshmi, Cause and effect of environmental pollution - Air, Water, Noise & Light. Pak. J. Biotechnol. 14(1): 109-113 (2017).

Su, Y., A. Mennerich, and B. Urban, Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. Bioresour. Technol. 105: 67-73 (2012).

Wu, T.Y., 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. J. of Environ. Manage. 91(7): 1467-1490 (2010).

Wang, L., Y. Li, P. Chen, M. Min, and Y. Chen, Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Bioresour. Technol. 101: 2623-2628 (2010).