Malaysian Journal of Microbiology, Vol 14(2) Special Issue 2018, pp. 145-151

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Post-treatment of palm oil mill effluent (POME) using freshwater green microalgae

Kah Aik Tan[1], Norhashimah Morad[1]*, Ismail Norli[1], Japareng Lalung[1], Wan Maznah Wan Omar[2]

[1]School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia.
[2] School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
Email: nhashima@usm.my

ABSTRACT

Aims: The effectiveness of microalgae in the post-treatment of palm oil mill effluent (POME) was being investigated for colour removal and COD reduction.
Methodology and results: Raw POME, obtained from a local palm oil mill and treated with anaerobic and aerobic processes for 50 days and 16 days of hydraulic retention time (HRT) respectively, was then used in the phycoremediation study. Three different species of microalgae (*Ankistrodesmus falcatus*, *Scenedesmus* sp. and *Chlorella* sp.) were inoculated in a culture media which contained 20%, 40% and 60% dilution of POME. The pH of the treated POME sample was not adjusted and fixed at the original pH of about pH 8-9. The growth of the microalgae was determined every 2 days based on their chlorophyll concentration. *Chlorella* sp. showed the best adaptation and grew well in all dilutions of the treated POME sample and subsequently chosen for remediation of the POME sample without any dilution.
Conclusion, significance and impact of study: Chemical oxygen demand (COD) and colour removal of POME were determined every 2 days. *Chlorella* sp. performed well with COD reduction and colour removal of 67.87% and 53.26%, respectively.

Keywords: Ankistrodesmus falcatus, Chlorella sp., green microalgae, palm oil mill effluent, *Scenedesmus* sp.

INTRODUCTION

Malaysia is one of the world's largest producer and exporter of palm oil as the Malaysian palm oil industry has grown rapidly over the years. Palm oil sludge or palm oil mill effluent (POME) is a type of wastewater which is generated from palm oil milling activities (Bashir *et al*., 2016). It contains a high concentration of chemical oxygen demand (COD) of 45,500-65,000 mg/L, biochemical oxygen demand (BOD) of 21,500-28,500 mg/L, total nitrogen (TN) of 500-800 mg/L, total phosphates (TP) of (94-131 mg/L), oil and grease (OG) of 1077-7582 mg/L and about 40,500 mg/L of total solid (TS) (Wu *et al*., 2010; Lam and Lee, 2011; Jalani *et al*., 2016). It requires multiple stages of treatment before being discharged into watercourse, due to the extremely high content of organic pollutants. If POME is discharged without any treatment, it will cause a very serious environmental pollution.

Anaerobic treatment method was found to be the best treatment method at the primary stage due to the high load of organics in POME. Many palm oil industries in Malaysia employ this type of treatment process at their primary stage treatment process. For the tertiary or polishing stage, many research works had reported that biological aerated filter (BAF) (Damayanti *et al*., 2011), ultra-filtration (Wu *et al*., 2007), membrane bioreactor process (Rotaru, #339) (Cheng *et al*., 2010) and adsorption (Shavandi *et al*., 2012) showed positive results for POME treatment. However, high capital costs and long hydraulic retention time (HRT) are the major disadvantages for these types of treatment processes. In addition, in some of the common conventional treatment facilities, fluctuations in the operating and raw POME concentrations cause the effluent released to be above the permissible limits set by the Department of Environment (DOE), Malaysia (Kamyab *et al*., 2013). Therefore, an extensive research work is currently underway to look for alternative post-treatment methods for POME.

Microalgae play an important role in self-purification of natural waters and are also able to remove a wide range of pollutants such as heavy metals, phosphorus, nitrogen etc. from wastewater (Arbib *et al*., 2012; Hongyang *et al*., 2011; Wang and Lan, 2011; Wang *et al*., 2014). Because of this ability, it offers an alternative post-treatment solution for many types of organic wastewater such as agricultural wastewater, domestic wastewater or industrial wastewater (Vairappan and Yen, 2008). Recent researches have determined that the substances in POME are able to support the growth of microalgae. POME is rich in degradable organic matter and contains the two fundamental components, nitrogen and phosphorus, which are needed for microalgae growth (Ahmad *et al*., 2003; Barsanti and Gualtieri, 2014).

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Microalgae are able to utilize the carbon and nutrient sources in POME for their growth and thus removing these contaminations (Ahmad *et al*., 2003).

Although the utilization of algae in various types of wastewater has increased over the years, reports on the use of microalgae for POME post-treatment are still limited. In this study, three different species of microalgae (*Ankistrodemus falcatus*, *Chlorella* sp. and *Scenedesmus* sp.) were isolated from different sources. These three strains were assessed for their growth rate in different dilutions of POME. The strain which showed the best adaptation in POME was then chosen to determine its efficiency in colour removal and COD reduction of POME.

MATERIALS AND METHODS

Wastewater collection

The raw POME used in this study, originated from a palm oil mill in Penang, Malaysia. The collected raw POME was pre-treated with two stages of anaerobic and aerobic processes with 50 days and 16 days of hydraulic retention time (HRT), respectively, to reduce the dark colour of POME. This pre-treatment enables the light to penetrate through POME and allows the migroalgae to grow. About 450 mL of the pre-treated POME was collected every day and stored in a refrigerator at 4 °C to limit the activity of the biodegradation process of the microorganisms. The characteristics of the raw POME and the pre-treated POME were analyzed and listed in Table 1.

Microalgae strains

Two single colonies strains of microalgae (*Scenedemus* sp. and *Chlorella* sp.) were isolated from Harapan Lake, Universiti Sains Malaysia. Another strain of microalgae, *Ankistrodesmus falcatus* was obtained from the School of Biological Sciences, Universiti Sains Malaysia. Each of the microalgae strain was inoculated in 1 L of Bold's Basal Medium (BBM) as a stock culture. All of the strains were agitated at 100 rpm on an orbital shaker under 12: 12 h (light: dark).

Bold's basal medium

A 1 L of stock media contained the following ingredients: 25 g/L $NaNO_3$, 7.5 g/L $MgSO_4 \cdot 7H_2O$, 7.5 g/L K_2HPO_4, 2.5 g/L $CaCl_2 \cdot H_2O$, 2.5 g/L NaCl, 11.42 g/L H_3BO_3, 50 g/L $EDTA.Na_2$, 31 g/L KOH, 4.98 g/L $FeSO_4 \cdot 7H_2O$, 1.0 mL concentrated HCL and 1.0 mL trace elements solution. The trace elements solution contained 8.82 g/L $ZnSO_4$, 1.44 g/L $MnCl_2 \cdot 4H_2O$, 1.59 g/L $CuSO_4 \cdot 5H_2O$, 0.71 g/L MoO_3, 0.49 g/L $Co(NO_3) \cdot 4H_2O$. The ingredients were diluted with 1L of distilled water and autoclaved for 15 minutes at 121 °C.

Isolation and cultivation conditions

Nutrient agar plates were prepared to cultivate the microalgae sample. A 1% of agar was mixed with BBM and autoclaved at 121 °C for 15 min to prepare the agar plates. A series dilution of samples was spread on the BBM agar plates and cultured under 2400±100 Lux of light intensity at room temperature 32±1 °C. After one week, the single colonies were streaked onto another agar plate. This procedure was repeated several times until a single strain of microalgae was obtained.

A 10 mL of each single strain of microalgae were inoculated in 250 mL Erlenmeyer flask which contained 100 mL of autoclaved BBM, and shaken at 100 rpm. All the cultures were maintained at a temperature of 32±1 °C with light and dark period of 12 h each at 2400±100 Lux of light intensity.

Experimental condition

A 10% of each microalga was inoculated in 250 mL Erlenmeyer flasks. Each of the flask contained 150 mL of wastewater media with different dilutions (20%, 40%, and 60%) of wastewater to BBM. The pH of the samples was not adjusted. All the flasks containing samples were covered with cotton wool and shaken at 100 rpm and cultivated at room temperature (32±1 ºC) with 2400±100 Lux of light intensity for 12 h of light/dark period. The chlorophyll concentrations, which indicates the growth of the microalgae, were determined every two days, from day-1 to day-16. The microalgae that survived were chosen to be used in the remediation of POME study.

Morphological study

All three microalgae species were identified based on the morphological examination of the individual cells under microscope. All three isolated colonies and the morphology of each microalgae species (before and after cultivation in treated POME sample) were photographed at magnification 40x using Nikon Eclipse (E200) microscope, equipped with Q-imaging digital camera and Image-Pro Express 6.0 software.

Growth rate study

A 1 mL from each sample was withdrawn from the flasks every two days. The sample was centrifuged at 10,000 rpm for 5 min to obtain the cell pellet. The supernatant was discarded. A 1 mL of 90% of methanol was added to the pellet. The mixture was stored at 4 °C in a dark condition for one hour before it was centrifuged again for another 5 min at 10,000 rpm to separate the supernatant and the cell pellet. The absorbance reading of the supernatant was then measured using Shimadzu UV-spectrophotometer (Model-UV 16001 PC) with wavelength 652 nm and 665 nm (Ritchie, 2008). The chlorophyll a concentration was calculated using equation (1) (Ritchie, 2008),

$$(1) conc.of\ chlorophyll\ a\ (mg/L) = -8.0962\ \lambda_{652nm} + 16.5169\ \lambda_{665nm}$$

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

The growth of the microalgae was determined based on the chlorophyll content of each strain. The specific growth rate, μ, is calculated according to equation (2) where N_1 and N_2 are the concentrations of chlorophyll a at time t_1 and t_2 respectively (He *et al*., 2016).

$$(2)\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1}$$

Phycoremediation of POME

A 5 mL of each sample was drawn every two days and filtered using Watchman Cellulose Nitrate Membrane Filter (0.45 μm of pore size) under vacuum. The filtered liquid sample was collected. The percentage of COD reduction and colour removal of the collected liquid sample were determined according to the standard methods for the examination of water and wastewaters (American Public Health *et al*., 1998). The removal efficiency for colour removal and the COD reduction were calculated using equation (3) below,

$$(3)removal\ efficiency\ (\%) = \frac{C_i - C_f}{C_i} \times 100\%$$

where, C_i and C_f is the initial and final reading of colour (Pt/Co) and COD (mg/L) respectively.

RESULTS AND DISCUSSION

Characteristics of POME

Anaerobic and aerobic treatment processes were carried out as pre-treatment processes for the raw POME after it was collected from the palm oil mill. The HRT of the anaerobic and aerobic process were 50 days and 16 days respectively. The characteristics of the raw POME, anaerobic treated POME and aerobic treated POME are listed in Table 1.

Table 1: Characteristics of POME.

Parameters	Raw POME	Anaerobic treated POME	Aerobic treated POME
Ph	4-4.5	7-7.5	8-9
Colour (Pt/Co)	-	-	3000-4500
COD (mg/L)	70,000-80,000	15,000-16,000	600-1000

Morphological and characteristics of microalgae

The three microalgae were screened and selected from each of the green single colony on the agar plate. The morphology of those strains was observed under a microscope with 40× magnification. The morphology of *Ankistrodesmus falcatus* is displayed in Figure 1(a). This type of microalgae is unicellular or colonial chlorophyte. The microalgae cells are curved or slightly crescent in shape. The cell length is around 25 to 62 μm and the cell width is around 1.2 to 4.3 μm.

Figure 1(b) shows the morphology of *Chlorella* sp. The microalgae cells are spherical in shape. The diameter of each microalgal cell is about 2-10 μm. The cells of this microalgae are without flagella and they are green in colour.

The morphology of *Scenedesmus* sp. is showed in Figure 1(c). The cells of this strain are arranged either linearly or in a zigzag pattern. The colonies of the cells are formed in a group. Around 2 or 4 cells of this microalgae usually will attach side by side. The cells of the microalgae are normally elliptical or spindle in shape with 11-18 μm long and 3.5-7 μm wide.

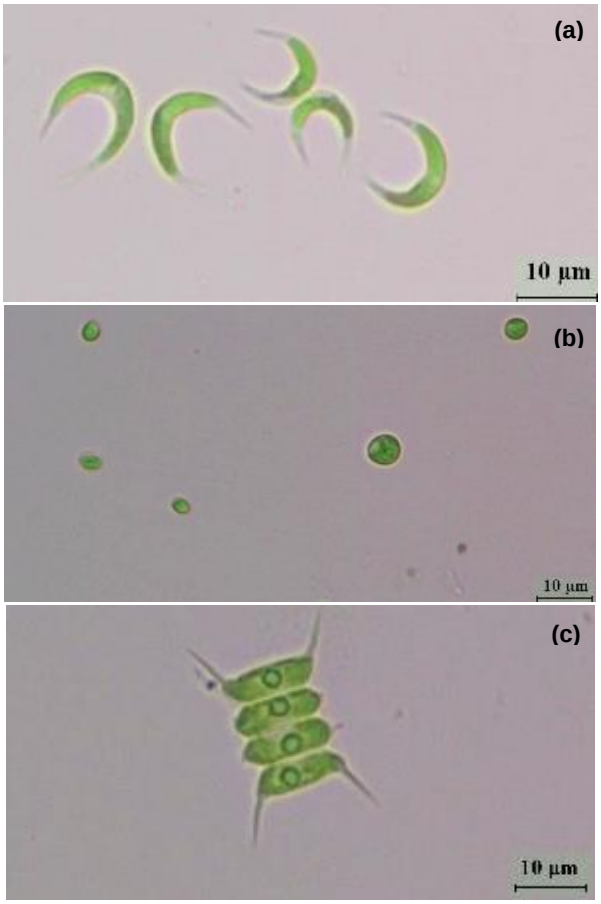

Figure 1: Morphology of (a) *Ankistrodesmus falcatus*, (b) *Chlorella* sp. and (c) *Scenedesmus* sp. with magnification of 40×.

Microalgae growth

The growth of the three microalgae were determined based on the concentration of chlorophyll a. The growth of *Ankistrodesmus falcatus, Chlorella* sp. and *Scenedesmus* sp. in the culture media which contained 20%, 40% and 60% of treated POME, are shown in Figure 2(a), 2(b) and 2(c), respectively. All three species grow well in the media

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

with 20% dilution of pre-treated POME. Both *Chlorella* sp. and *Scenedesmus* sp. attained their maximum growth on 12th day with 6.340 mg/L and 1.500 mg/L of concentration of chlorophyll a, respectively. The specific growth rate was 0.1297 mg/Lday for *Chlorella* sp. and 0.0243 mg/Lday for *Scenedesmus* sp. But, *A. falcatus* showed maximum growth on 16th day with 2.975 mg/L of concentration of chlorophyll a and specific growth rate with 0.0652 mg/Lday.

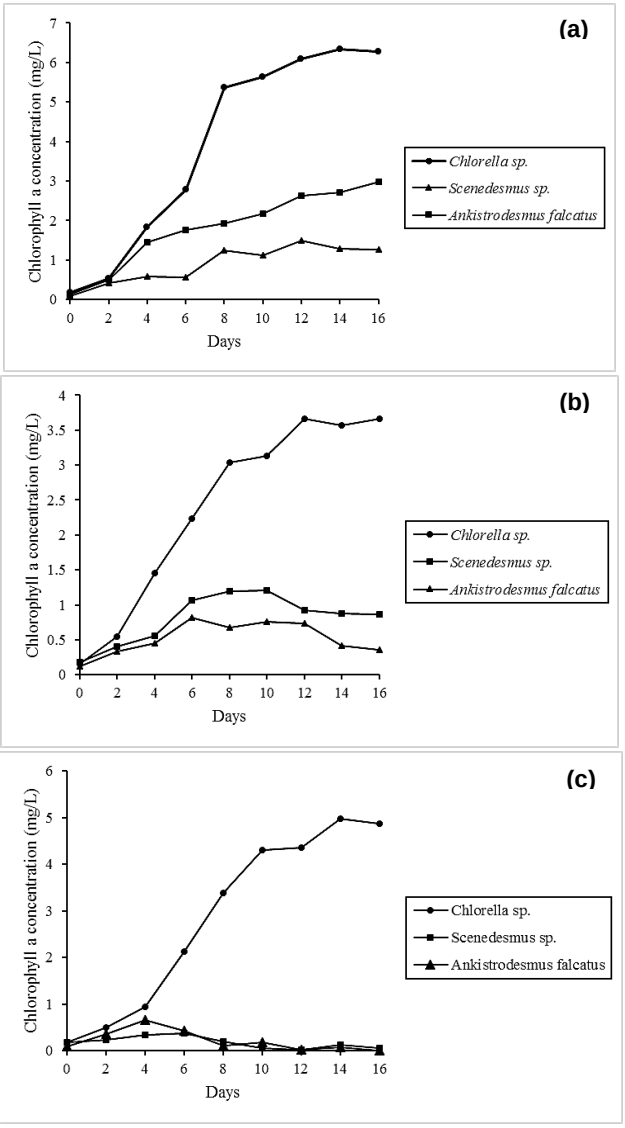

Figure 2: The growth of the three species of green microalgae in media which contain of (a) 20%, (b) 40% and (c) 60% of POME.

However, the increase of percentage of pre-treated POME in the media decreased light penetration. This caused the decrease of the growth rate of both *A. falcatus* and *Scenedesmus* sp., as the volume of treated POME increased in the media. Light is an essential resource which often limits the growth rate of microalgae. George *et al*. (2014) had investigated the growth of *A. falcatus,* and found that it is highly influenced by the light intensity (George *et al*., 2014). The results showed *A. falcatus* grew well as light intensity is increased (George *et al*., 2014). Liu *et al*. (2012) also reported that the highest biomass production of *Scenedesmus* sp. was obtained at the highest light intensity (Liu *et al*., 2012).

Among these three microalgae, *Chlorella* sp. showed good adaptation even in the lowest dilution, i.e. 60% volume of pre-treated POME in media. Some of the microalgae species will be in mixotrophic condition whereby photoautotroph and heterotroph apply simultaneously under ample light condition (Bhola *et al*., 2011). They are able to switch between autotrophic and heterotrophic growth regime based on the culture condition. Many researchers had observed that *Chlorella* sp. can tolerate high concentration of wastewater even in poor light penetration (Li *et al*., 2011). *Chlorella* sp. can use both metabolic pathways to utilize the carbon source and organic matter in the wastewater. Under poor light penetration condition, the *Chlorella* sp. will switch to heterotrophic growth regime.

pH is another factor which will affect the growth of microalgae. Different species of microalgae can tolerate different values of pH. In this study, the pH of pre-treated POME was very high, at about pH 9. This might have caused *Scenedesmus* sp. and *A. falcatus* to not able to grow well in the pre-treated POME sample.

Morphology of microalgae in POME media

The morphology of each microalgae species after being cultivated in the media which contained POME was monitored under microscope with magnification of 40×. Figure 3(a) displays the morphology of *Chlorella* sp. after 16 days of cultivation period. The cells were clumped together after the 16th day of cultivation. This might be caused by the production of extracellular polymeric substances (EPS) to protect the cells from extreme conditions, in this case, from POME. Figure 3(b) and 3(c) show the morphology of *Scenedesmus* sp. and *Ankistrodesmus* sp. Many dead cells of *Scenedesmus* sp. and *A. falcatus* (circled in red) were found within the 16 days of cultivation as both species could not tolerate the extreme condition of pre-treated POME, which has high pH and poor light intensity.

COD reduction and colour removal

Chlorella sp. was chosen in the subsequent study on COD reduction and colour removal of POME (without dilution). Figure 4 shows the growth rate of *Chlorella* sp. in POME. *Chlorella* sp. achieved its maximum growth rate on 12th day with 3.6195 mg/L of chlorophyll concentration with the specific growth rate 0.2878 mg/Lday. The chlorophyll concentration decreased after day 12th and this indicated the death phase in POME for this strain.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Figure 5(a) and 5(b) show the efficiency of COD reduction and colour removal of *Chlorella* sp. respectively within 16 days. The highest removal percentage of colour removal and COD reduction was obtained at day 10th with 53.26% and 67.87%, respectively. There was slight removal of colour and COD reduction of the control samples (without autoclave) which could be due to the degradation activity caused by the bacteria present in POME.

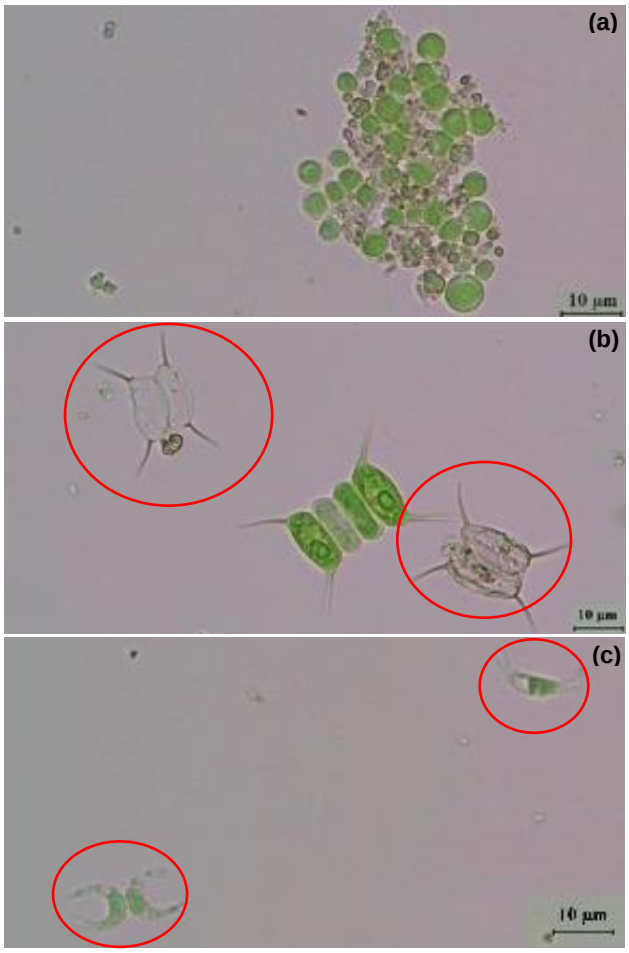

Figure 3: The morphology of (a) *Chlorella* sp., (b) *Scenedesmus* sp. and (c) *A. falcatus* after the 16 days of cultivation.

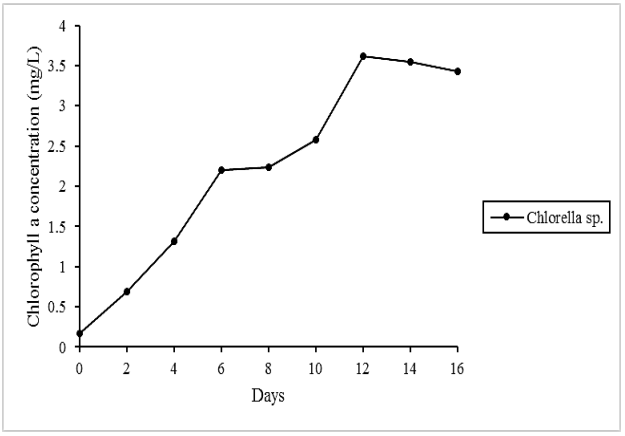

Figure 4: The growth of *Chlorella* sp. in POME (without dilution) within 16 days.

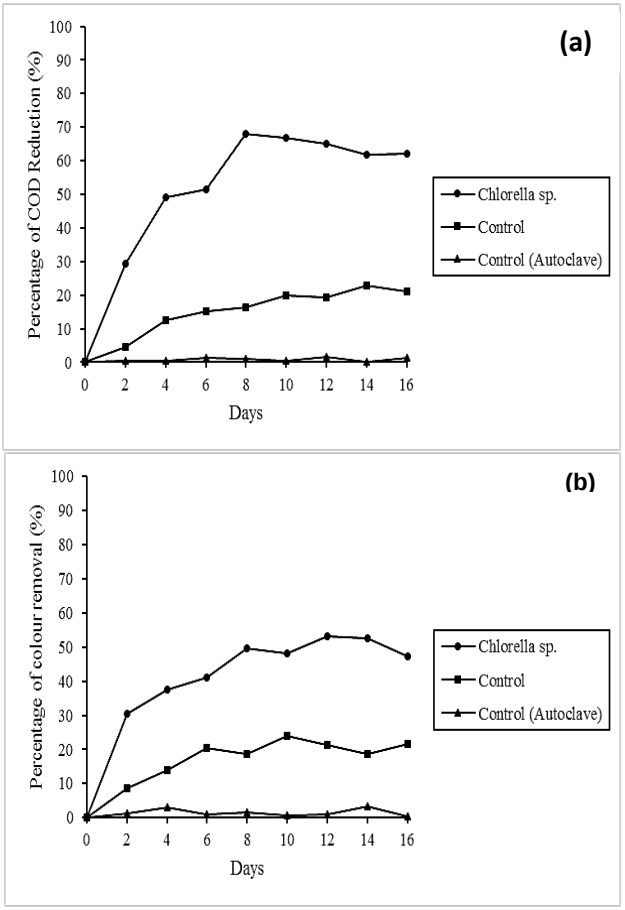

Figure 5: The percentage efficiency of (a) COD reduction and (b) colour removal of POME (without dilution) for 16 days.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

CONCLUSION

Compared to another two strains, *A. falcatus* and *Scenedesmus* sp.*, Chlorella* sp. could adapt well in POME even though the POME sample was poor in light penetration due to being dark brown in colour. *Chlorella* sp. has a potential to be used for post-treatment of POME since it showed a good result on COD reduction and colour removal of POME with about 67.87% and 53.26%, respectively.

Ankistrodesmus falcatus and *Scenedesmus* sp. showed good adaptation in media which contained low concentration of POME. *Chlorella* sp. performed the best amongst the three strains. However, there are several parameters such as pH, temperature, light intensity etc. which need to be considered, in order to obtain the optimum growth of these microalgae and enhance the good performance of POME post-treatment process.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support from Universiti Sains Malaysia in the form of Research University Grant No. 1001/PTEKIND/811291, which has resulted in this paper.

REFERENCES

Ahmad, A. L., Ismail, S., and Bhatia, S. (2003). Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination* **157(1), 87-95.**

American Public Health, A., American Water Works, A. and Water Environment, F. (1998). Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF, Washington, D.C.

Arbib, Z., Ruiz, J., Alvarez, P., Garrido, C., Barragan, J. and Perales, J. A. (2012). *Chlorella stigmatophora* for urban wastewater nutrient removal and CO2 abatement. *International Journal of Phytoremediation* **14(7), 714-725.**

Barsanti, L. and Gualtieri, P., (2014). Algae: Anatomy, Biochemistry, and Biotechnology. CRC press.

Bashir, M. J., Han, T. M., Wei, L. J., Aun, N. C. and Amr, S. S. A. (2016). Polishing of treated palm oil mill effluent (POME) from ponding system by electrocoagulation process. *Water Science and Technology* **73(11), 2704-2712.**

Bhola, V., Desikan, R., Santosh, S. K., Subburamu, K., Sanniyasi, E. and Bux, F. (2011). Effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris*. *Journal of Bioscience and Bioengineering* **111(3), 377-382.**

Cheng, J., Zhu, X., Ni, J. and Borthwick, A. (2010). Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. *Bioresource Technology* **101(8), 2729-2734.**

Damayanti, A., Ujang, Z. and Salim, M. R. (2011). The influenced of PAC, zeolite, and *Moringa oleifera* as biofouling reducer (BFR) on hybrid membrane bioreactor of palm oil mill effluent (POME). *Bioresource technology* **102(6), 4341-4346.**

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T. and Mishra, S. (2014). Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus*–A potential strain for bio-fuel production. *Bioresource technology* **171, 367-374.**

He, N., Sun, X., Zhong, Y., Sun, K., Liu, W. and Duan, S. (2016). Removal and biodegradation of nonylphenol by four freshwater microalgae. *International Journal of Environmental Research and Public Health* **13(12), 1239.**

Hongyang, S., Yalei, Z., Chunmin, Z., Xuefei, Z. and Jinpeng, L. (2011). Cultivation of *Chlorella pyrenoidosa* in soybean processing wastewater. *Bioresource Technology* **102(21), 9884-9890.**

Jalani, N. F., Aziz, A. A., Wahab, N. A., Hassan, W. H. W. and Zainal, N. H. (2016). Application of palm kernel shell activated carbon for the removal of pollutant and color in palm oil mill effluent treatment. *Journal of Earth, Environment and Health Sciences* **2(1), 15-20.**

Kamyab, H., Ponraj, M., Soltani, M., Din, M. F. M., Eva, S. and Mohamad, A. M. R. (2013). Micro-macro algal mixture as a promising treatment for POME discharge and it is potential use as animal feed stock enhancer. *Jurnal Teknologi* **68(5), 1-4.**

Lam, M. K. and Lee, K. T. (2011). Renewable and sustainable bioenergies production from palm oil mill effluent (POME): Win–win strategies toward better environmental protection. *Biotechnology Advances* **29(1), 124-141.**

Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., Zhu, J. and Ruan, R. (2011). Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology* **102(8), 5138-5144.**

Liu, J., Yuan, C., Hu, G. and Li, F. (2012). Effects of light intensity on the growth and lipid accumulation of microalga *Scenedesmus* sp. 11-1 under nitrogen limitation. *Applied Biochemistry and Biotechnology* **166(8), 2127-2137.**

Ritchie, R. J. (2008). Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica* **46(1), 115-126.**

Shavandi, M. A., Haddadian, Z., Ismail, M. H. S. and Abdullah, N. (2012). Continuous metal and residual oil removal from palm oil mill effluent using natural zeolite-packed column. *Journal of the Taiwan Institute of Chemical Engineers* **43(6), 934-941.**

Vairappan, C. S. and Yen, A.M. (2008). Palm oil mill effluent (POME) cultured marine microalgae as supplementary diet for rotifer culture. *Journal of Applied Phycology* **20(5), 603-608.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Wang, B. and Lan, C. Q. (2011). Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent. *Bioresource Technology* **102(10), 5639-5644.**

Wang, M., Kuo-Dahab, W.C., Dolan, S., Park, C. (2014). Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, *Chlorella* sp. and *Micractinium* sp., in wastewater treatment. *Bioresource Technology* **154, 131-137.**

Wu, T. Y., Mohammad, A. W., Jahim, J. M. and Anuar, N. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. *Journal of Environmental Management* **91(7), 1467-1490.**

Wu, T. Y., Mohammad, A. W., Md. Jahim, J. and Anuar, N. (2007). Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: Effect of pressure on membrane fouling. *Biochemical Engineering Journal* **35(3), 309-317.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538