Phycoremediation of Treated Palm Oil Mill Effluent (TPOME) using *Nannochloropsis* sp. Cells Immobilized in the Biological Sodium Alginate Beads: Effect of POME Concentration

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The use of freely suspended cells of microalgae culture to treat wastewater is of current global interest because of their effective photosynthetic uptake of pollutants, carbon dioxide sequestration, and biomass production for desirable high value-products. Biomass immobilization is a promising option to overcome the harvesting problem that is encountered when using free-cells upon completion of the wastewater treatment process. In this study, *Nannochloropsis* sp. cells were immobilized in sodium alginate beads to eliminate the harvesting limitation. The microalgal beads were further cultivated in treated palm oil mill effluent (TPOME) for removal of chemical oxygen demand (COD). The effect of POME concentration on COD removal and microalgal cells growth was investigated, respectively. It was found that the maximum biomass concentration of 1.23 g/L and COD removal of 55% from 10% POME were achieved after 9 days. An increment of POME concentration did not cause any improvement to the treatment efficiency due to the inhibitory effect of high initial COD of POME on the biomass concentration and was further responsible for low COD removal. The immobilized cells showed a systematic growth, demonstrating that the beads are biocompatible as immobilization carrier. In conclusion, the immobilized microalgal cells could be a viable alternative technology system for POME treatment as well as biomass production.

Keywords: Wastewater pollution and treatment; Microalgae; Biomass growth; COD; Removal efficiency; Sustainability

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INTRODUCTION

Malaysia is the world's largest producer of palm oil, which is one of the world's leading agricultural commodities. There are more than 400 mills throughout the country. Most of the mills have generated around 800 tonnes/day of palm oil mill effluent (POME) resulting from the palm oil processing (Halim *et al.* 2016). POME has a high concentration of organic matter identified as chemical oxygen demand (COD) and biochemical oxygen demand (BOD_3) which are up to 51000 and 25000 mg/L, respectively (Zainal *et al*. 2017). The accidental discharge of untreated or treated POME into the environment could result in the eutrophication of water bodies and accelerate the dwindling of freshwater resources at a high rate. Eventually this can result in low levels of dissolved oxygen, a high mortality rate of zooplankton, depletion of aquatic life, and murkiness of water systems. Conventional technologies for POME treatment are sometimes still not capable of reducing the level of pollutants sufficiently to meet the discharge standards set by the department of environment (DOE) (Kamyab *et al*. 2014). Therefore, a highly efficient, cheap, and environmentally

friendly approach is required for the POME treatment.

Microalgae are microscopic photosynthetic organisms that can be found in marine ecosystems and freshwater. They consume organic matter and nutrients including nitrogen and phosphorus present in the wastewater for their growth (Chinnasamy *et al*. 2010). According to Emparan *et al*. (2019), phycoremediation is defined as the use of algae to remove or transform pollutants, including nutrients and toxic chemicals from wastewater, and removal of CO_2 from waste air accompanied by biomass production. Microalgae in the form of suspended free-cells of culture has been used for phycoremediation of wastewater including POME for removal of BOD, COD, and nutrients (Rajkumar and Takriff 2015). Pollutants present in the wastewater can be assimilated by the algae and can accumulate in the biomass, since the algal cell walls are porous which allow free passage of molecules and ions in aqueous solution (Lage *et al*. 2018). Recent studies have shown that the biomass of suspended free-cells from microalgae culture of the treated POME can be used to produce high-value products such as biodiesel (Selmani *et al*. 2013) and feedstock in aquaculture industry (Selvam *et al*. 2015).

However, recovery of suspended free-cells from microalgae biomass using the treated wastewater including POME is one of the challenges during a phycoremediation process. The suspended free-cells culture is the condition of microalgae living cells that move independently within the bottles containing medium under a condition to ensure uniform cells distribution (Katarzyna *et al*. 2015; Emparan *et al*. 2019). Therefore, the application of immobilized microalgal cells is a good approach to overcome the harvesting problem. The immobilized cells are living microalgal cells that are prevented from flowing freely away from their original location to all parts of the medium (Katarzyna *et al*. 2015; Emparan *et al*. 2019). In comparison to the other immobilization methods (adsorption, covalent binding, cross-linking, encapsulation), entrapment of microalgal cells in natural and synthetic gel polymers presents advantages of higher nutrients/products diffusion rates, more environmentally friendly character, and greater stability (Das and Adholeya 2015; Eroglu *et al.* 2015).

Natural polymers such as alginate are the most commonly used materials to immobilize the microalgal cells before the phycoremediation of wastewater. The advantages of using natural polymers as immobilizing carriers are their non-toxic, transparent, permeable, hydrophilic, nutrient enriched, environmentally friendly, and bio-compatible nature; they also have high product diffusion rates and produce less hazardous waste upon completion of the process (Shi et al. 2007; Zhang *et al*. 2008; Moreno-Garrido 2008; Eroglu *et al*. 2015; Sumithrabhai *et al*. 2016; Emparan *et al*. 2019). Since the immobilized beads have a larger size than suspended free-cells, a simpler method by sieving/netting can be employed to harvest the beads from water without requiring high energy input (Lam and Lee 2012) compared to suspended free-cells. Solvents are used to dissolve the beads and then oven-dried (where this process is also done in suspended free-cells). Also, the immobilized microalgal cells exhibited higher removal efficiency of pollutants from wastewater as compared to the suspended free-cells of microalgal culture (Zeng *et al.* 2013a).

Therefore, this study investigated the potential of microalgae, *Nannochloropsis* sp. cells immobilized in sodium alginate beads for phycoremediation of treated POME. So far, there have been no such studies carried out for removing pollutants from POME using immobilized *Nannochloropsis* sp. cells. In fact, the main objective of this study is to treat the POME without any chemical usage and apply the biological method *via* microalgae sodium alginate beads.

EXPERIMENTAL

Algal Cultivation

The species of microalgae used in this study was *Nannochloropsis* sp., which was supplied by the CSIRO Microalgae Research Centre (Tasmania, Australia). The microalgal cell was cultivated in F/2 culture media under the axenic condition at the algal research laboratory (Department of Chemical and Environmental Engineering, Universiti Putra, Malaysia). The suspended free-cells of microalgae culture was maintained by routine sub-culturing to prolong their life and to expand the cells number as stock media. The microalgal cells were harvested prior to the immobilization procedure.

Preparation of Sodium Alginate-Immobilized Microalgae

Sodium alginate beads with immobilized microalgal cells were obtained from sodium alginate-microalgae suspension. Approximately 4% (w/v) of sodium alginate solution was prepared and autoclaved at 121 °C and 15 psi for 15 min. Then, the suspended free-cells in microalgae culture were collected from the stock medium. They were then mixed with the 4% of sodium alginate solution at a ratio of 1:1 (v/v) to produce a 2% suspension of sodium alginate-microalgae. The mixture was extruded through a sterile disposable 5 mL syringe and a 25 gauge hypodermic needle into 0.3 M calcium chloride ($CaC1_2$). About 520 microalgae beads were produced from 40 mL of microalgae sodium alginate solution for sample A1 as shown in Table 1. All experiments were performed in duplicates. Therefore, the total amount of microalgae beads used in this batch studies were about 3120. The microalgae beads were left in $CaC1_2$ solution overnight for hardening stage. After that, the beads were washed with sterile distilled water. Strict aseptic precautions were adopted throughout the immobilization procedure.

Collection of POME

The POME used in this study was obtained from Sime Darby Palm Oil Plantation Sdn. Bhd., Labu, Negeri Sembilan (Malaysia). The effluent was obtained after sterilization, extraction, and purification. The raw POME was further treated through a series of ponding systems including an acidification pond, followed by an anaerobic pond, aerobic pond, and final pond. The POME sample was collected from the final pond/algae pond and stored in 20 L plastic containers with proper labeling. The sample was stored in the fridge at 4 °C to prevent contamination and limit the biodegradation process by bacterial activity. The POME was initially filtered using a filter paper to remove bacteria and suspended in sludge prior to microalgae cultivation experiment.

Treatment of POME Using Microalgal Cells Immobilized in Sodium Alginate Beads

Effect of POME concentration on microalgal cells growth and COD removal efficiency

The phycoremediation studies were performed in a batch culture using *Duran bottles* at the algal research laboratory, Department of Chemical & Environmental Engineering, Universiti Putra Malaysia. Three different concentrations of POME (10%, 25%, and 100 % POME) were prepared in 500 mL bottles as shown in Table 1. The selection of the ranges were based on Ding *et al.* (2016). In this study, the ratio of microalgae beads to each POME sample was fixed at 1:5 (v/v). In this respect, about 520 microalgae beads were put into each 200 mL POME sample. The fluorescence light energy and aeration using a magnetic stirrer at 100 rpm were continuously supplied to all samples. The bottles were stuffed with cotton

plugs to filter and allow movement of air into and out of the bottles thus preventing the entry of fungal spores or bacteria. Furthermore, the cotton plugs are cheap and can be re-autoclaved and recycled for use in the next experimental work. All the experiments were carried out at room temperature. The well-mixed microalgae beads suspension were collected and the microalgal cell growth was measured every 3 days and up to 9 days of the treatment period. The COD of each POME sample on Day 0 and Day 9 were also measured using the following Eq. 1,

$$COD\ (\%) = [(C_o - C_i)/C_o] \times 100\% \quad (1)$$

where C_o (mg/L) and C_i (mg/L) are the mean values of COD concentrations at initial time t_o (day) and time t_i (day), respectively.

Algal Analysis

Morphological examination using a microscope

This method is the fast and easy way to examine the condition of immobilized microalgal cells in the sodium alginate beads. Five microalgae beads were collected and dissolved using 0.1 M sodium citrate ($Na_3C_6H_5O_7$) solution. Approximately 1.0 mL of the dissolved immobilized-microalgae beads was added to one drop of oil red stain. The mixture was vortexed for about 1.0 min and centrifuged at 5 RCF for about 5 min. The supernatant was removed, and about 1.0 mL of 85% propylene glycol was added to wash the pellet. The mixture was again vortexed and centrifuged at 5 RCF for about 5 min and the supernatant was removed again. The procedure was repeated using 50% propylene glycol. After the supernatant was removed, distilled water was added and vortexed to homogenize the pellet. Each sample was then ready to be examined under the JVC Color Video Camera microscope (Leica PTE Ltd, Singapore) with a magnification of 40x and 100x.

Scanning electron microscope (SEM)

The morphological structure of microalgal cells, sodium alginate microalgae beads, and blank sodium alginate beads were investigated using Jeol JSM 6400 SEM (Hitachi, Tokyo, Japan). Prior to microscopic observation, each sample was processed through several processes, including fixation, washing, and dehydration. After that, each sample was ready to be viewed under the SEM.

Optical density (OD) and biomass concentration

The OD of microalgal cells was measured using a GENESYS™ 10S UV-Vis spectrophotometer at an optimum wavelength of 600 nm (OD_{600}). A calibration curve was prepared by plotting OD against biomass concentration (in dry weight, g/L). Prior to analysis, five microalgae beads were dissolved using 2 mL of 0.1 M $Na_3C_6H_5O_7$ solution and placed into a clean UV-cuvette to measure the absorbance value. For suspended free-cell microalgae culture (standard graph), 2 mL of the sample was used. Distilled water was used as a blank sample. The OD was recorded and biomass concentration was obtained from the calibration curve.

Wastewater Analysis

Chemical oxygen demand (COD)

In this study, the COD measurement was carried out according to the standard method 5220C (Closed Reflux, Titrimetric) (APHA 2017). Initially, about 2.5 mL of POME sample was added into a COD vial. Another test vial filled with 2.5 mL of distilled water

was prepared as a blank sample. After that, each sample was mixed with 1.5 mL of potassium dichromate ($K_2Cr_2O_7$) (9.05 mol/L), followed by 3.5 mL of sulfuric acid (H_2SO_4) (18.01 mol/L). The vials were mixed well and placed in the COD reactor at 150 °C for 2 h. After 2 h, all the vials were allowed to cool to reach room temperature. Each sample was then placed into a conical flask and added with 3 drops of ferroin indicator. Then, each sample was titrated with 0.10 M ferrous ammonium sulfate (FAS) solution until the color change from pale blue-green into reddish-brown. The volume of FAS used was recorded. The COD of the each was calculated based on the following Eq. 2,

$$COD\ \left(\frac{mg}{L}\right) = \frac{(A-B)\times 0.1\times 8000}{2.5\ mL} \quad (2)$$

where *A* is the volume of FAS (mL) used in blank sample and *B* is the volume of FAS (mL) in POME sample.

Turbidity

The turbidity of each sample was determined based on HACH Method 10047 (Attenuated Radiation Method) using a DR/4000 spectrophotometer. Firstly, the spectrophotometer was turn on and Hach Program: 3750 at 860 nm was selected. After that, about 10 mL of distilled water was put into a vial as blank. Then, about 10 mL of the sample was put into other vials. Each vial was put into the spectrophotometer, and the turbidity reading (FAU) was measured and recorded.

Hydrogen ion concentration

The pH of the sample was determined based on Model 3505 user guide using pH meter (Brand JENWAY).

Statistical Analysis

All the experiments were carried out in duplicate. The analysis of variance (ANOVA) SPSS version 20 was used to determine the statistical significance among treatments at $p < 0.05$.

Table 1. Composition of POME and Sodium Alginate Microalgal Cells Solution

Ratio of sodium alginate algal cell beads to POME (v/v)	Dilution factor	Initial COD (mg/L)	Initial pH	Label	Volume of distilled water (mL)	Volume of raw POME (mL)	Total volume of POME (mL)	Volume of algal cell culture (mL)	Volume of sodium alginate solution (mL)	Number of sodium alginate algal cells beads
1:5	10% POME	252.5 ± 31.8	8.74 ± 0.3	A1	180	20	200	20	20	520
				A2						520
1:5	25% POME	662.5 ± 123.7	8.66 ± 0.3	B1	150	50	200	20	20	520
				B2						520
1:5	100% POME	2100 ± 282.8	8.59 ± 0.3	C1	-	200	200	20	20	520
				C2						520

RESULTS AND DISCUSSION

Immobilization of Microalgal Cells in the Sodium Alginate Beads

Immobilization of the microalgal cells using sodium alginate was carried out to enhance the harvesting and efficiency of POME treatment. As mentioned in the methodology section, the microalgal cell culture was mixed with the sodium alginate solution at a ratio of 1:1 (v/v) and extruded using a syringe to form a spherical shape of the beads in $CaC1_2$ solution. The microalgal beads were then left overnight for the hardening stage. The procedure was repeated without the addition of microalgal cells culture with blank sodium alginate bead samples.

The images of blank and sodium alginate microalgal cell beads are shown in Fig. 1. According to Fig. 1, it was observed that the blank sodium alginate beads were transparent in color. After mixing with microalgal cell culture, the color of beads changed into light green. The color of the cultivated microalgal beads changed to darker green after the POME treatment for 9 days, hence demonstrating that microalgal cells were successfully grown inside the beads.

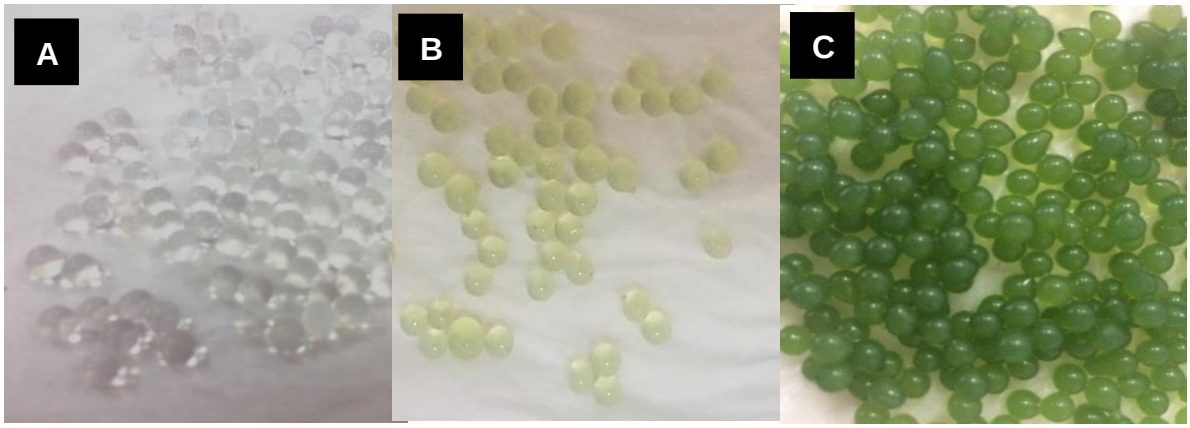

Fig. 1. Image of (A) blank sodium alginate beads; (B) immobilized sodium alginate microalgal cell beads before treatment; and (C) immobilized sodium alginate microalgal cell beads after 10% POME treatment.

Morphological examination using a microscope

The analysis was carried out using a microscope to determine the condition of microalgal cells in the beads after the immobilization process. The oil red stain is a natural lipophilic dye with known spectral properties. As shown in Fig. 2, the microalgal cells exhibited green and red color where its lipid had been dyed with the red stain. The structure and appearance of microalgal cells did not show any changes before and after immobilization into the beads. This showed that the immobilization will not affect the morphological structure and metabolic activity of microalgae. Eventually, microalgae can survive and grow in the beads.

Scanning electron microscope (SEM)

The morphological structure of immobilized cells, blank sodium alginate beads, and sodium alginate microalgal cell beads were examined using a scanning electron microscope (SEM). The outer and inner surfaces of blank sodium alginate beads are shown in Fig. 3. The outer surface of blank sodium alginate bead was smooth. The blank sodium alginate

beads were then cut into half to observe the cross-section of the inner surface and some pores with size 300 nm to 500 nm were observed. The images of immobilized microalgal cell beads are shown in Fig. 4. From the outer surface, it was observed that there were insoluble particulates attached to microalgal cells. The particulates might be the insoluble sodium alginate powder. From the cross-sectional surface, the microalgal cells were bonded with each other and entrapped by the sodium alginate. The pores were used to exchange materials such as CO_2, oxygen, nutrient sources, and metabolites inside and outside the capsules (Zeng *et al*. 2013b).

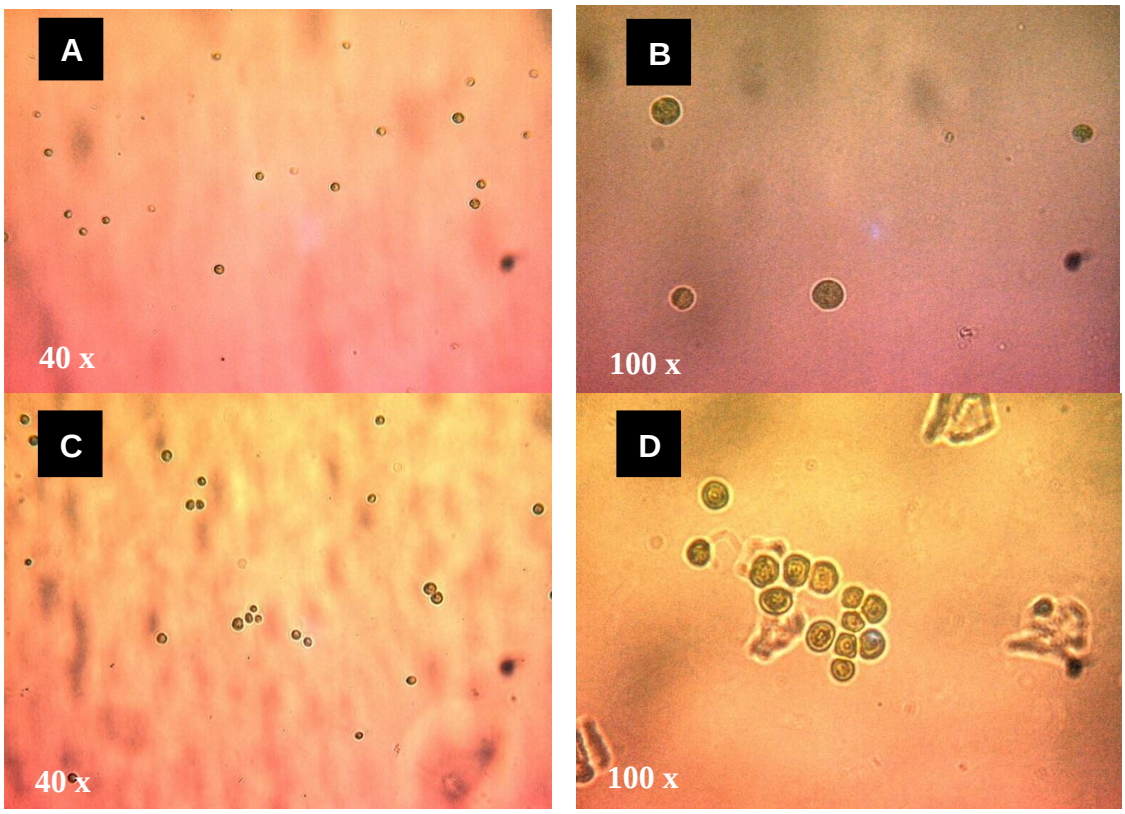

Fig. 2. *Nannochloropsis* sp. cells (A) without beads; (B) without beads; (C) with sodium alginate microalgal cell beads; and (D) with sodium alginate microalgal cell beads

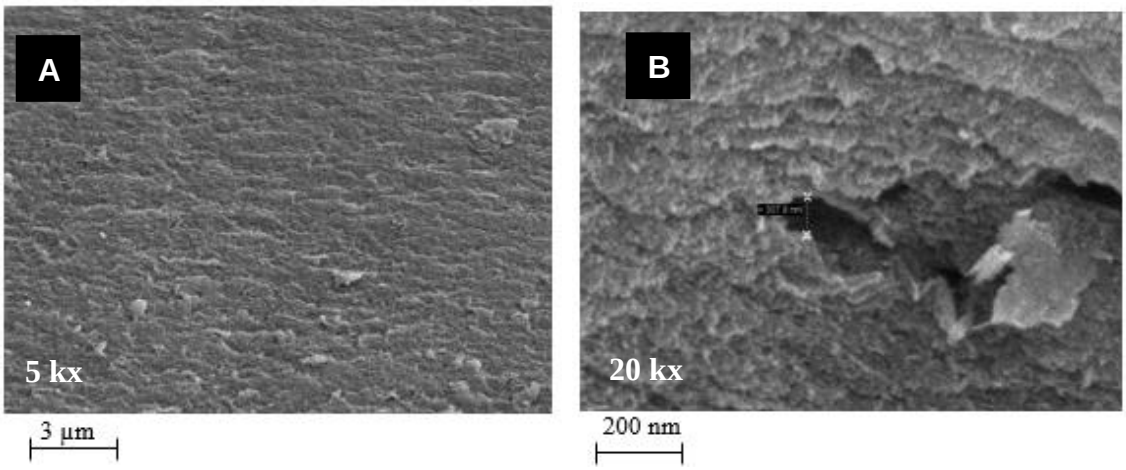

Fig. 3. Blank sodium alginate bead: (A) outer surface; and (B) inner surface pore

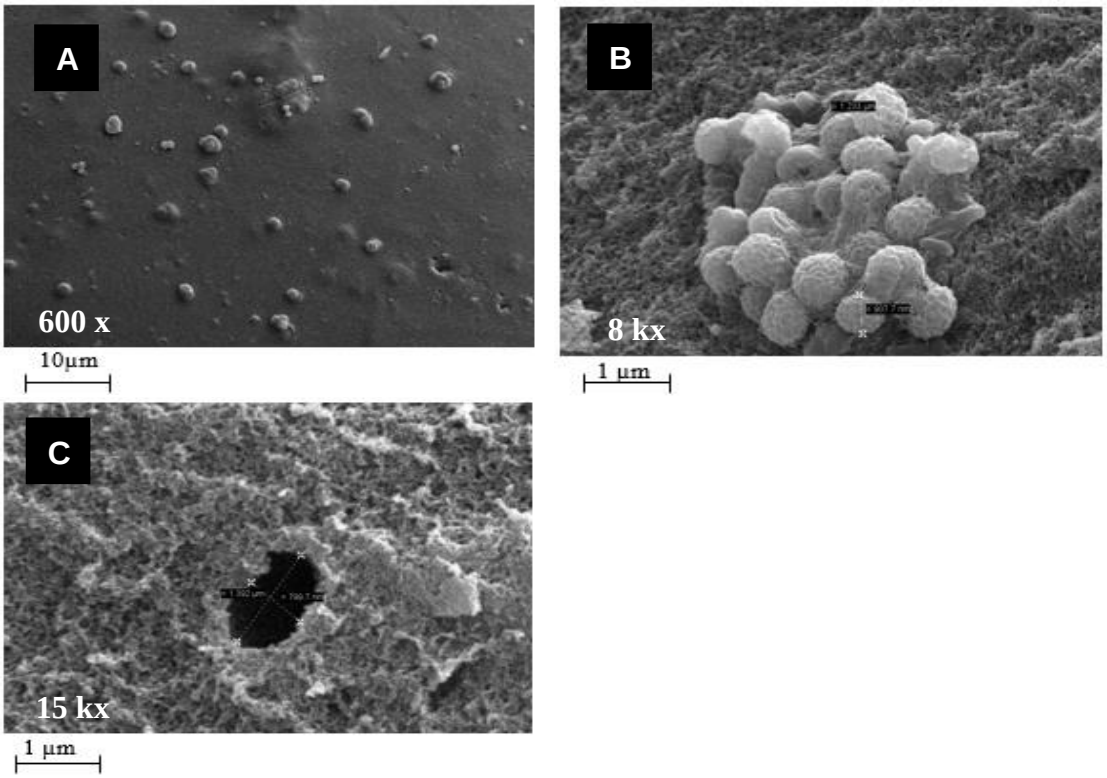

Fig. 4. Immobilized sodium alginate microalgal cell beads: (A) outer surface; (B) inner surface; and (C) inner surface pore.

POME treatment using microalgal cells immobilized in the sodium alginate beads
Effect of POME concentration on biomass concentration of immobilized microalgal cells

The immobilized microalgal cells cultivated in each POME sample indicated a similar growth curve, as shown in Fig. 5. The standard growth curve was obtained from the authors' previous study using *Nannochloropsis* sp. cells cultivated in the synthetic F/2 media with maximum biomass concentration up to 1.05 ± 0.01 g/L ($p < 0.05$) (Tang 2016). According to Fig. 5, the biomass concentration increased with increasing time, and the trend was consistent for all samples. This indicated that the immobilized microalgal cells were able to carry out cell division and photosynthesis in spite of insufficient growth space inside sodium alginate beads. It was found that the immobilized cells had higher biomass concentration compared to the suspended cells samples. They displayed a better viability and growth rate than suspended free-cells samples. For all the samples, the microalgal cell growth was slightly slower during the first 3 days due to the adaptation period to the new environment. There was a rapid growth trend from Day 3 to 9, indicating the cells were adapted to the POME media and started their exponential phase. In 100% POME sample, the microalgal beads were completely dissolved into the raw POME after Day 3 of the treatment period. It was also observed that the growth of microalgal cells was significantly decreased up to Day 3 and the beads were fully dissolved at Day 6 (non-viable cells were observed). Hence 100% raw POME was not suitable for the application of immobilized sodium alginate microalgae beads. The microalgal cells in 100% POME attained the maximum biomass concentration of 0.1050 mg/L on Day 6. For 10% and 25% POME samples revealed a consistent growth trend from Day 0 to 9. In 10% POME, the biomass concentration of microalgal cells was increased from 0.24 g/L to 1.23 g/L ($p < 0.05$). In

25% POME, the biomass concentration was increased from 0.24 g/L to 1.07 g/L ($p < 0.05$) in 9 days. This indicated that the higher concentration of POME lowered the maximum biomass concentration of microalgal cells.

Cultivation of suspended free-cells of *Nannochloropsis* sp. cells in 10% POME with an initial COD of 165 mg/L achieved a maximum growth microalgal cells at OD_{625} of 0.4 throughout the treatment period (Hadiyanto *et al*. 2017). It has been shown that suspended free-cells of *Chlamydomonas* sp. UKM 6 grown in various POME concentrations accomplished maximum biomass concentration of microalgae up to 0.917 g/L (Wang *et al*. 2010). In addition, this study indicated that a higher biomass concentration of 1.23 g/L (with OD_{600} of 0.6) was obtained using 10% POME with an initial COD of 252.5 mg/L using sodium alginate microalgal cell beads than Hadiyanto *et al*. (2017) and Wang *et al*. (2010). The reason for this was attributed to the protection of immobilized microalgal cells by a sodium alginate membrane from direct exposure to high POME concentration. In this respect, the microalgal cell growth under high POME concentration, microalgae species as bioremediator, and immobilized form of microalgae cells were all explored in this study. These findings showed that the immobilized cells used in the study were more robust against high concentrations of POME, as indicated by a higher microalgal growth cell (with OD_{600} of 0.6 and biomass concentration of 1.23 g/L) than the aforementioned suspended free-cells of *Nannochloropsis* sp. (with OD_{625} of 0.4) and *Chlamydomonas* sp. UKM 6 (with biomass concentration of 0.917 g/L). These findings showed that the immobilized cells used in the study can be a viable approach to produce greater biomass production than suspended free-cells of *Nannochloropsis* sp. and *Chlamydomonas* sp. UKM 6.

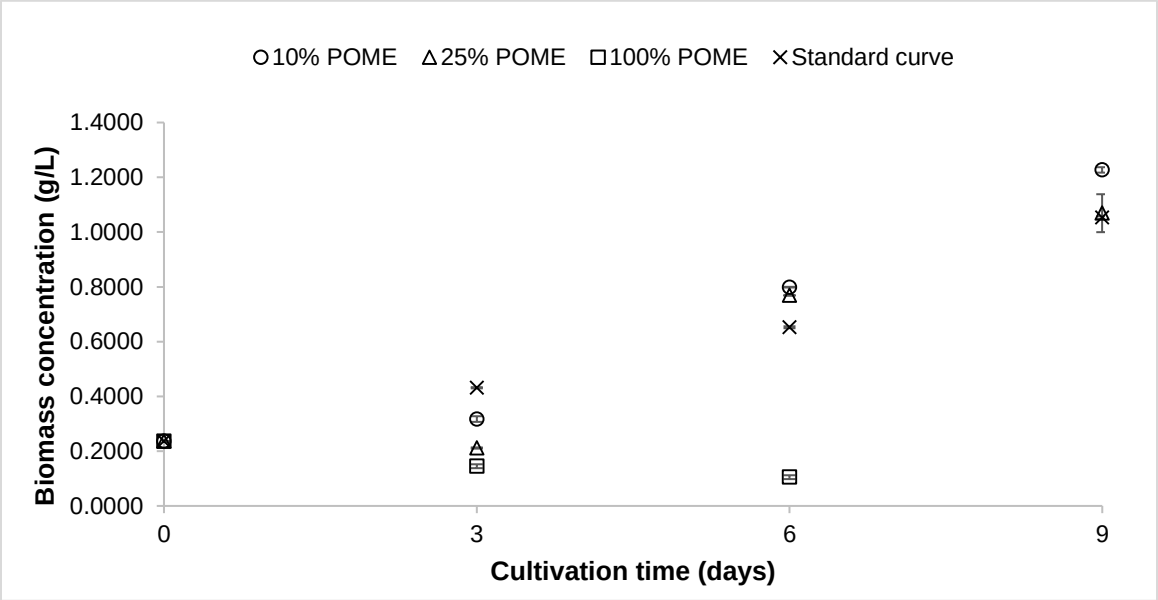

Fig. 5. Biomass concentration against time with various POME concentrations

Effect of POME concentration on COD removal efficiency

The chemical oxygen demand (COD) is the measure of oxygen equivalent of organic matter of a sample that is susceptible to be oxidized by a strong chemical oxidant. It is a vital parameter to identify the efficiency of microalgae to remove organic matter in wastewater. Figure 7 shows the COD of various concentrations of POME on Days 0 and 9. A noticeable reduction of COD in 9 days was observed, which were 55% for 10% POME

($p < 0.05$) and 50% for 25% POME ($p > 0.05$), respectively. The *Nannochloropsis* sp. cells were in the heterotrophic growth metabolism, which consumed the organic matter as a carbon source for growth resulting in the reduction of COD over the treatment period. Organic carbon is one of the important nutrients for building up microalgal cells. In fact, most of the composition of microalgal cells was made up of carbon (Selmani *et al*. 2013). These findings indicated that the immobilized cells were very effective in removing COD at 10% POME with initial COD of 252.5 mg/L. The COD removal at 25% POME with initial COD of 662.5 mg/L was comparable to that of 252.5 mg/L, while at 100% POME with initial COD of 2100 mg/L, the COD removal was drastically decreased. These findings also showed that the organic matter/organic carbon at a certain concentration inhibited the COD removal from POME and microalgae cells growth. The COD was increased within 3 days in the sample with 100% POME due to the dissolution of the microalgae beads, resulting in leakage of cells that become carbon contributor to POME (Maria and Anggraini 2018). As aforementioned in the previous section, the cells growth of microalgal was decreased after 3 days of treatment. This might be due to the microalgal cells not being able to remain in viable form, resulting in a decaying process to occur and thus reducing the biomass concentration. According to Borg *et al*. (1997), the increase of COD in 100% POME was also due to the increase in organic input from the decaying algae. As mentioned before, the growth of microalgae cells was significantly decreased up to Day 3 and the beads were fully dissolved at Day 6, as shown in Fig. 5 (non-viable cells were observed).

Many studies focusing on various species of microalgae cultivated in POME media for COD removal have been reported. Hadiyanto *et al.* (2017) used suspended free-cells of *Nannochloropsis* sp. to remove COD from 10%, 30%, and 50% POME samples. Lower removal of COD up to 34% was achieved as compared to the study using immobilized *Nannochloropsis* sp. cells. In this study, the highest COD removal of 55% was found in the 10% POME with initial COD of 252 mg/L. Suspended free-cells of *Chlamydomonas* sp. UKM 6 reduced COD within the range of 8% to 29% from various concentrations of POME (Ding *et al.* 2016), which was lower than immobilized *Nannochloropsis* sp. cells reported in this study. This demonstrated that the immobilized *Nannochloropsis* sp. cells (with OD_{600} of 0.6 and biomass concentration of 1.23 g/L) more robust and efficient against POME with an initial COD of 252.5 mg/L, as indicated by a higher removal of COD (55%) than aforementioned suspended free-cells of *Nannochloropsis* sp. (34%) and *Chlamydomonas* sp. UKM 6 (29%). Kamyab *et al.* (2014), reported that a micro-macro algal mixture was capable in removing maximum 71% of COD from the POME sample with a lower initial concentration of COD, which also in the line of this study. In this respect, the different species of robust microalgae can also be mixed to achieve higher removal efficiency of COD from POME in the future. The successful use of suspended free-cells of *Nannochloropsis* sp. cells and other microalgal species to remove COD from POME has also been reported by several researchers (Ahmad *et al.* 2015; Ammar *et al.* 2018; Hadiyanto *et al.* 2014; Rajkumar and Takriff 2015; Selvam *et al.* 2015; Shah *et al.* 2016). Based on the aforementioned previous reports, the biomass concentration and COD removal efficiency were influenced by POME concentration and type of microalgae species (suspended free-cells or immobilized cells) to be used for POME treatment.

According to Maria and Anggraini (2018), the cellular respiration of microalgal cells resulting in organic carbon assimilation process in the growth media. In this process, the organic compound and oxygen were used as an electron donor and final electron acceptor, respectively. The cellular respiration was a source of energy for treatment and biosynthesis in dark conditions. The microalgal cells will consume inorganic carbon sources such as CO_2

and organic carbon derived from the media for their growth during mixotrophic culture mode (Perez-Garcia *et al.* 2010).

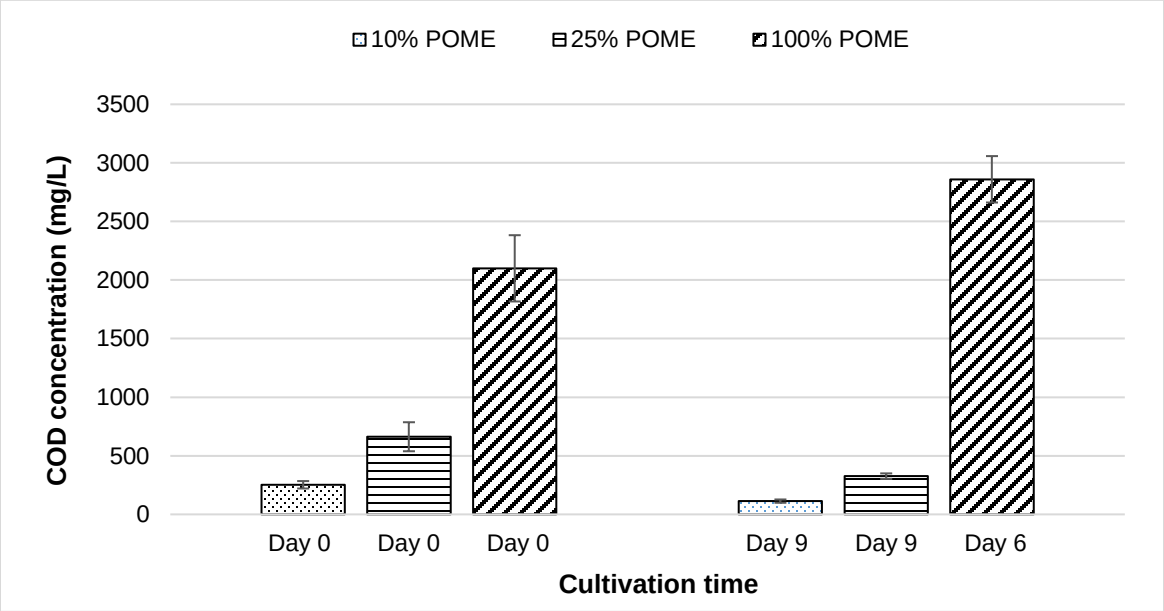

Fig. 6. COD in various POME concentrations on Day 0 and Day 9

Physical examination

The physical examination of POME and immobilized microalgal cells in the sodium alginate beads were carried out in 3 days intervals. The images of the samples were taken as shown in Figs. 8, 9, and 10. The green color intensity in the sodium alginate beads was increased from Day 0 to Day 9. The green color resembles the chlorophyll intensity in the cell, which is essential for the photosynthesis process. In Fig. 9, the sodium alginate microalgae beads were completely dissolved in 100% POME media after Day 3. These findings indicated that the beads cannot sustain the high concentration of POME (100% POME with initial COD of 2100 mg/L), as mentioned before. Besides that, the continuously decreases of biomass concentration from 0.24 g/L (Day 0) to 0.11 g/L (Day 0) samples suggested that the immobilized microalgal cells were not suitably cultivated in 100% POME. The 100% POME (25.5 ± 6.36 FAU) had more darkness of color followed by 25% POME (9 ± 0.05 FAU) and lastly 10% POME (2 ± 0.05 FAU). The dark color of POME limits and blocks the penetration of light through the bottles to the immobilized microalgal cells in the POME media (Ding *et al.* 2016; Hariz and Takriff 2017). Therefore, the very high POME concentration and the absence of light hinders photosynthesis, causing death to the microalgal cells. This explained the reason why 100% POME exhibited the lowest cell growth rate followed by 25% POME and lastly 10% POME. Based on the obtained results, 10% POME with initial COD of 252.5 mg/L was selected as the sample for the future studies involving optimization of COD removal and microalgal cell growth.

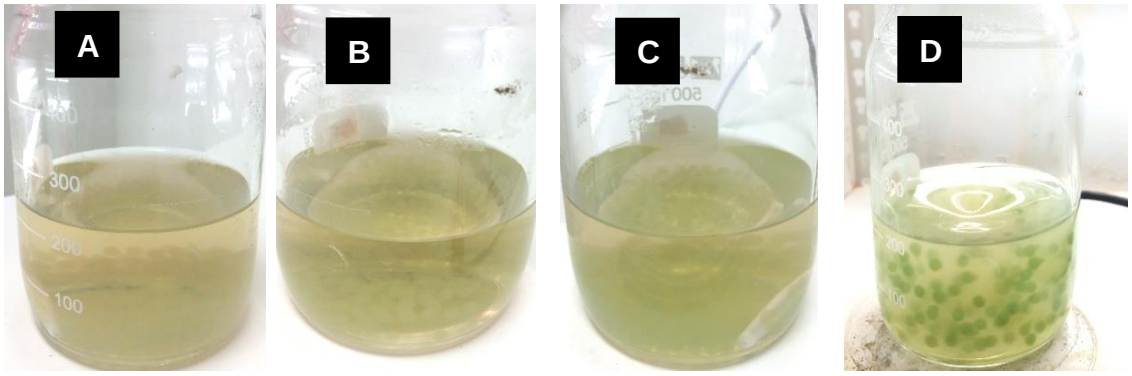

Fig. 7. Microalgal cell beads in 10% POME on Day (A) 0; (B) 3; (C) 6; and (D) 9

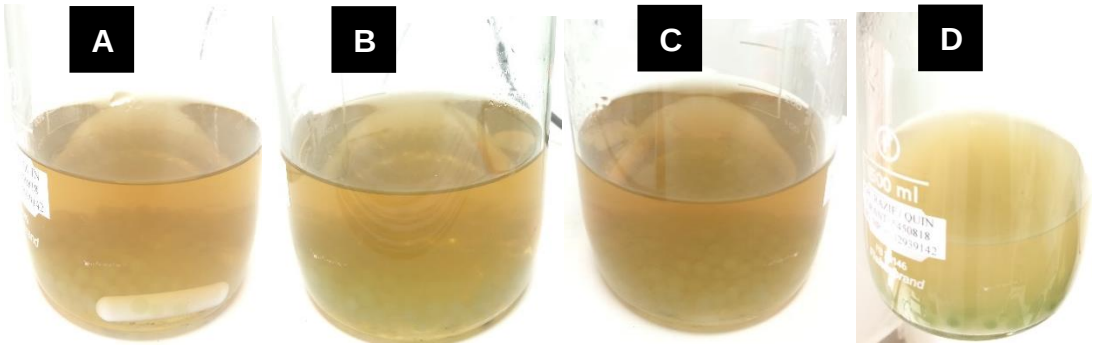

Fig. 8. Microalgal cell beads in 25% POME on Day (A) 0; (B) 3; (C) 6; and (D) 9

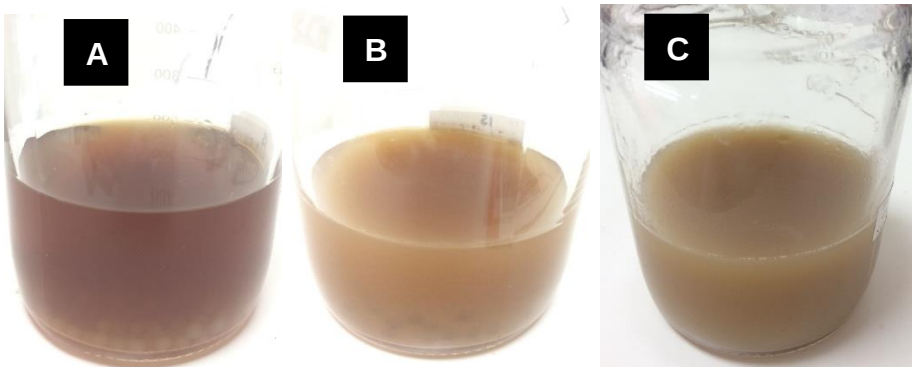

Fig. 9. Microalgal cell beads in 10% POME on Day (A) 0; (B) 3; and (C) 6

CONCLUSIONS

1. Pollutants such as organic matter/organic carbon contained in palm oil mill effluent (POME) can be used as a viable source of nutrients for removal of chemical oxygen demand (COD) using sodium alginate beads immobilized with *Nannochloropsis* sp. cells.
2. The immobilized microalgal cells were able to adapt and consume organic matter and

nutrient content in POME for their metabolic and photosynthetic activities.

3. The removal efficiency of COD and biomass concentration of immobilized microalgal cells were influenced by variations in POME concentrations such as 10%, 25%, and 100%.
4. The biomass concentration of immobilized microalgal cells achieved a maximum of 1.23 g/L at the end of treatment.
5. The immobilized microalgal cells performed efficiently with 55% COD removal from 10% POME using fixed 520 sodium alginate microalgae beads.

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