

Integrated Palm Oil Mill Effluent Treatment and CO₂ Sequestration by Microalgae (Rawatan Bersepadu Efluen Kilang Kelapa Sawit dan Pemerangkapan CO₂ oleh Mikroalga)

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ABSTRACT

Malaysian economy relies on palm oil industries as a driver for rural development. However, palm oil mill effluent (POME) that is generated from palm oil processing stages causes major environmental challenges. Before being released to the environment, POME treatment is crucial to comply with standard discharge limit. Microalgae have demonstrated excellent potential for phycoremediating POME and capturing CO₂. In this study, local microalgae isolate such as *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 were used for POME treatment in 21 days with different inoculum sizes (5%, 10% and 15%). In addition, an integrated treatment process was performed by taking the treated POME supernatant for cultivation of *Chlorella* spp. UKM 2, *Chlorella sorokiniana* UKM 3 and *Chlorella vulgaris* for CO₂ sequestration study. Different CO₂ concentrations (5%, 10% and 15%) were used and the experiments were carried out in 10 days under continuous illumination. The results showed that among two species involves in POME treatment, *Chlamydomonas* sp. UKM 6 showed a great potential to remove pollutant such as COD (56%), nitrogen (65%) and phosphorus (34%). The biomass after POME treatment and CO₂ biofixation content high lipid (90 mg lipid/g biomass) which can be the potential source for biodiesel production. In CO₂ sequestration study, *C. sorokiniana* UKM3 able to takes up to 15% CO₂ with CO₂ uptake rate of 273 mgL⁻¹d⁻¹. In this study, the integrated system of POME treatment and CO₂ sequestration were feasible using microalgae.

Keywords: *Chlorella* spp.; *Chlamydomonas* sp.; CO₂ capture; effluent treatment; microalgae

ABSTRAK

Ekonomi Malaysia bergantung kepada industri kelapa sawit untuk pembangunan kawasan luar bandar. Walau bagaimanapun, efluen kilang kelapa sawit (POME) yang terhasil pada peringkat pemprosesan minyak kelapa sawit menyebabkan masalah besar kepada alam sekitar. Sebelum dilepaskan ke alam sekitar, rawatan POME sangat penting untuk mematuhi had pelepasan piawai yang telah ditetapkan. Mikroalga telah menunjukkan potensi yang amat baik bagi tujuan bioremediasi POME dan pemerangkapan CO₂. Dalam kajian ini, penciran mikroalga tempatan iaitu *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 telah digunakan untuk merawat POME dalam jangka masa 21 hari menggunakan saiz enap cemar yang berbeza (5%, 10% dan 15%). Di samping itu, proses rawatan bersepadu dijalankan dengan mengambil supernatan POME yang telah dirawat untuk inokulasi *Chlorella* spp. UKM 2, *Chlorella sorokiniana* UKM 3 dan *Chlorella vulgaris* bagi kajian pemerangkapan CO₂. Kepekatan CO₂ yang berlainan (5%, 10% dan 15%) telah digunakan dan uji kaji telah dijalankan selama 10 hari di bawah pencahayaan yang berterusan. Keputusan kajian menunjukkan bahawa, antara dua spesies yang digunakan untuk rawatan POME, *Chlamydomonas* sp. UKM 6 menunjukkan potensi yang besar untuk menurunkan kadar COD (56%), nitrogen (65%) dan fosforus (34%). Biojisim selepas rawatan POME dan pemerangkapan CO₂ menunjukkan nilai lipid yang tinggi (90 mg lipid/g biomas) yang berpotensi menjadi sumber penghasilan biodiesel. Dalam kajian pemerangkapan karbon, *C. sorokiniana* UKM3 boleh memerangkap sehingga 15% CO₂ dengan kadar pemerangkapan CO₂ sebanyak 273 mgL⁻¹d⁻¹. Justeru, proses integrasi antara rawatan POME dan pemerangkapan karbon boleh dijalankan menggunakan mikroalga.

Kata kunci: *Chlorella* spp.; *Chlamydomonas* sp.; mikroalga; pemerangkapan CO₂; rawatan efluen

INTRODUCTION

In 1971, palm oil industry became a commercial commodity in Malaysia and the first plantation was developed at Tennamaran Estate in Selangor. This industry grow rapidly and currently Malaysia is the second largest producer and exporter of palm oil in the world after Indonesia which contributes 11% from the world's oils and fats production and 27% of export trade of oils and fats (Amin 2014).

In addition, the palm oil industry provides employment to more than half a million people and livelihood to an estimated one million people.

Although oil palm industry has been recognized for its contribution to Malaysian economic growth, this industry also contributed to environmental pollution caused by the production of by-products in large quantities during oil extraction process such as empty fruit bunches (EFB), oil

palm fronds (OPF), oil palm trunks (OPT) and liquid palm oil mill effluent (POME) which causes severe impact to environment and community (Singh et al. 2010). According to Environmental Quality (prescribed Premises) (Crude Palm Oil) Regulations 1977 (Department of Environment 1977), the requirement for POME to be released to the water stream should meet the regulatory standard of BOD (20 mg/L), COD (1000 mg/L), total solid (1500 mg/L), suspended solid (400 mg/L), oil and grease (50 mg/L) and total nitrogen (50 mg/L). The most significant pollutant from the industry is POME that it can cause air pollution such as GHGs emission and water pollution if there is no proper waste management implementation according to Environmental Quality Act 1978 (Environmental Quality (Clean Air) Regulations 1978).

There are a few method in treating POME and the most common methods that is adopted by 85% palm oil mill in Malaysia is using a conventional method that consist of anaerobic, aerobic and facultative ponds (Ahmad & Krimly 2014). This method is widely used due to its ability to treat large quantity of POME with low operating costs. However, the challenges with ponding system in which treatment process lead to greenhouse gas (GHGs) emission that can cause the global warming. In reality, the treated POME using this method sometimes does not meet the requirement of standard industrial discharge implemented by the Department of Environment (Wu et al. 2010).

To date, microalgae are considered as a new alternatives in biological wastewater treatment due to their effectiveness in treating effluent and CO₂ biofixation. POME have been used as nutrient source for microalgae cultivation since POME have enough nutrients to support microalgal growth. Microalgae such as *Chlamydomonas* sp. UKM 6 (Ding et al. 2016), *Chlorella sorokiniana* (Khalid et al. 2018), *Botryococcus brauni* (Nur et al. 2018) and mix culture of microalgae (Babua et al. 2017) have been cultivated in POME. Furthermore, algae biomass produced were useful in food industry, medical, biofertilizer, animal feed, biodiesel and biogas production. Compared with macroalgae, microalgae have a great potential as a biofuel renewable sources due to its fast growth rate and its ability to store lipid and carbohydrate in its cells (Demirbaş 2008). Thus, the use of microalgae offers three important advantages such as wastewater treatment, CO₂ sequestration and production of valuable products. Therefore, the aims of this work was to investigate the performance of locally isolated microalgae in an integrated system of POME treatment and CO₂ sequestration. In this study, *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 were used for POME treatment. Then, the supernatant after POME treatment was used as the cultivation media for the growth of *Chlorella* spp. UKM 2, *Chlorella sorokiniana* UKM 3 and *Chlorella vulgaris* for the objective of CO₂ biofixation. The integrated system of POME treatment and CO₂ biofixation intentions are to treat POME in an efficient way to meet the discharge standard with an additional values of CO₂ sequestration.

MATERIALS AND METHODS

The sample of POME was obtained from Sime Darby Palm Oil Mill, Tennamaram, Bestari Jaya, Selangor. The sample was taken from a clarifier overflow after an anaerobic digester. POME sample was kept in the cool room at 4°C to prevent any changes on its characteristics and should not keep more than two weeks to avoid excessive deterioration (Daud et al. 2013). The characteristics of POME are presented in Table 1.

TABLE 1. Characteristics of POME

Parameter	Units	Value
BOD ₃	mgL ⁻¹	675 ± 44
COD	mgL ⁻¹	2046 ± 167
Total phosphorus	mgL ⁻¹	292 ± 16
Total nitrogen	mgL ⁻¹	195 ± 6
Turbidity	NTU	460 ± 15
pH	-	7.96 ± 0.08

The microalgae strains used in this work were locally isolated strain, *Chlamydomonas* sp. UKM 6, *Chlorella* spp. UKM 8, *C. sorokiniana* UKM 2, as well as commercially obtained strains, *C. vulgaris*. *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 were used for POME treatment while *Chlorella* spp. UKM 2, *C. sorokiniana* UKM 3 and *C. vulgaris* were used for CO₂ sequestration study. Ten percent (v/v) of these strains were cultured in the Bold Basal's media (BBM) as stated by Ding et al. (2016). The microalgae were kept at ambient temperature at 30°C under continuous illumination (54 μmol m⁻² s⁻¹) and sparged with a constant air flow rate at 2000 mLmin⁻¹ for 21 days, followed by measurement of dry weight and optical density (OD).

Two hundred mL of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 were inoculated independently in 1800 mL of BBM. Then, microalgae species were cultured until the OD value reach 0.8. Different inoculum sizes (5%, 10% and 15% v/v) were inoculated into 2L Duran bottle containing POME. The cell concentration used as the starting point in each experiment is in the range of 0.08-0.1 g/L biomass dry weight. The cultures were kept at ambient room temperature under continuous illumination of 54 μmol m⁻² s⁻¹ at air flow rate of 2000 mLmin⁻¹ for 21 days. The microalgae cultivation up to 21 days was carried out to ensure the growth of microalgae reach stationary phase for efficient treatment of POME (Figure 1). The measurement of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were analyzed referring to HACH Method 8000, 10072 and 10127, respectively, according to the manufacturer protocol. Lipid was analyzed based on Bligh and Dyer extraction of total lipid (Bligh & Dyer 1959). Chlorophyll a (chl a) and carotenoid were analyzed according to Watada et al. (1976).

Then, the effluents (supernatant) from the POME treatment were used in CO₂ sequestration study with different concentration of CO₂ (5%, 10% and 15%) in the mixed air. Six hundred mL of each culture of *Chlorella* sp. UKM 2, *C. sorokiniana* UKM 3 and *C. vulgaris* were inoculated into 1400 mL of supernatant after POME treatment. The microalgae were kept at ambient room temperature under continuous illumination (54 μmol m⁻² s⁻¹) with the air flow rate at 2000 mLmin⁻¹ for 10 days, followed by measurement of COD, TP and biomass characterization. The maximum specific growth rate, μ_{max} (day⁻¹), of the microalgae growth was calculated using the Verhulst logistical model via (1) and (2). Equation (2) is the integral of (1) (Rao et al. 2009).

$$\frac{dx}{dt} = \mu_{\max} \cdot x \cdot \left(1 - \frac{x}{X_{\max}}\right) \quad (1)$$

$$X = \frac{X_{\max}}{1 + e^{-2 \cdot \mu_{\max} \cdot t}} \quad (2)$$

where x (mg L⁻¹) is the biomass concentration in the time-course; X_{\max} (mg L⁻¹) is the maximum biomass concentration; and μ_{\max} (day⁻¹) is the maximum specific growth rate. The calculation of μ_{\max} was conducted using the OriginPro 9 Program.

The maximum productivity of microalgae growth was derived using (3) (Rao et al. 2009),

$$P_{\max} = \frac{dx}{dt} = \frac{X_{\max} \cdot \mu_{\max}}{4} \quad (3)$$

where P_{\max} (mg L⁻¹ day⁻¹) is the maximum productivity of the microalgae.

The CO₂ fixation rate of microalgae growth in POME was determined by (4) (Tang et al. 2011),

$$R_{\text{co}_2} = C_c \cdot P_{\max} \cdot \frac{M_{\text{co}_2}}{M_c} \quad (4)$$

where C_c is the carbon content of the microalgal cells (% w/w); P_{\max} (mg L⁻¹ day⁻¹) is the maximum productivity of the microalgae; M_{co_2} is the molar mass of CO₂ (g mol⁻¹) and M_c is the molar mass of carbon (g mol⁻¹).

RESULTS AND DISCUSSION

Figure 1 shows the growth profile of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 at different inoculum sizes (5, 10 and 15% (v/v)) in POME. The profile was fitted with a modified logistic model (1) for identification of kinetics parameters such as maximum specific growth rate (μ_{max}), lag phase (λ) and R² as indicated in Table 2. It was observed that the maximum specific growth rate (μ_{max}) was obtained at inoculum size of 10% (v/v) at 0.285 day⁻¹ and 0.311 day⁻¹ for *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8, respectively. An increased inoculum size to 15% (v/v) resulted in slower growth rate due to limitation of nutrient sources due to an increased number of microalgae. A study by Holdmann et al. (2018) and Sforza et al. (2014) explained that the self-shading effects occurs with increased in microalgae cell concentration due to unstable light intensities in culture medium. Therefore, slow growth rate can be observed with increasing cell concentration (Table 2).

Then, the effluent after 21 days of POME treatment that consist of biomass of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 were harvested using centrifugation method before the supernatant were used as a growth media in CO₂ sequestration study. Two local isolates (*Chlorella* spp. UKM 2 and *C. sorokiniana* UKM 3) and one commercial species (*C. vulgaris*) were used in CO₂ sequestration study. Figures 2, 3 and 4 show the growth profile and its kinetic parameters of *Chlorella* spp. UKM 2, *C. sorokiniana* UKM 3 and *C. vulgaris* grew in different growth media (supernatant of *Chlamydomonas* sp. UKM 6 or *Chlorella* sp. UKM 8) and different concentration of CO₂ (5%, 10% and 15%) in a gas mixture. The CO₂ sequestration study utilized the treated POME as growth medium (cultured in the supernatant of *Chlamydomonas*

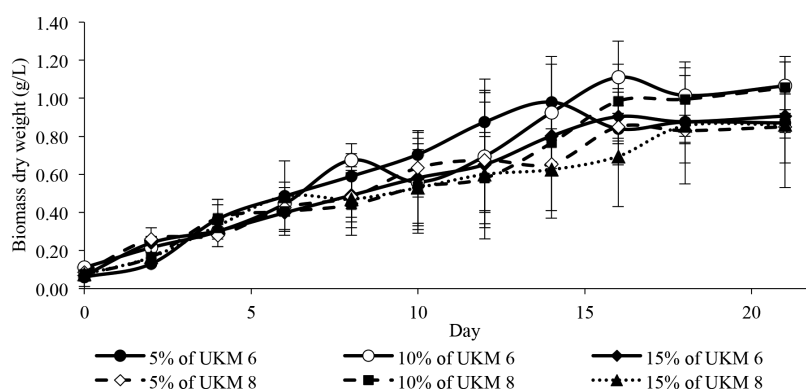


FIGURE 1. Growth profile of *Chlamydomonas* sp. UKM 6 and *Chlorella* sp. UKM 8 using three different inoculum sizes during POME treatment

TABLE 2. Kinetic growth parameter based on logistical model

Parameter	<i>Chlamydomonas</i> sp. (UKM 6)			<i>Chlorella</i> spp. (UKM 8)		
	Inoculum size (v/v)					
	5%	10%	15%	5%	10%	15%
μ_{\max} (day ⁻¹)	0.254	0.285	0.236	0.244	0.311	0.21
Lag phase (day)	1.2	0.9	-	0.1	1.3	-
R ²	0.891	0.832	0.821	0.809	0.886	0.917

sp. UKM 6 and *Chlorella* sp. UKM 8). *C. vulgaris* that grew in 5% CO₂ displayed the highest maximum specific growth rate (μ_{\max}) in the supernatant of *Chlamydomonas* sp. UKM 6 (0.7 day⁻¹) and *Chlorella* spp. UKM 8 (0.43 day⁻¹). The results obtained in this study shows that CO₂ concentration that exceed 5% corresponded to slightly low maximum specific growth rate only for *C. vulgaris* grew in both growth media. Similar results were obtained in previous study where *C. vulgaris* cultured in 15% CO₂ resulted in lower biomass productivity and maximum specific growth rate (Zheng et al. 2012). However, *Chlorella* spp. UKM 2 shows the highest specific growth rate in the supernatant of *Chlamydomonas* sp. UKM 6 (0.65 day⁻¹) and *Chlorella* spp. UKM 8 (0.54 day⁻¹) in 5% and 10% CO₂, respectively. Meanwhile, *C. sorokiniana* UKM 3 shows the highest specific growth rate in the supernatant of *Chlamydomonas* sp. UKM 6 (0.83 day⁻¹) and *Chlorella* spp. UKM 8 (0.64 day⁻¹) in 10% and 15% CO₂, respectively. The results obtained showed that all microalgae are able to survive in the CO₂ concentration up to 15%. It was shown in Table 3 that the overall CO₂ capture rate (R_{CO₂}) for each species are not the same due to the types of microalgae, nutrient sources and CO₂ concentration. *C. sorokiniana* UKM 3 displayed the highest CO₂ capture rate (R_{CO₂}) followed by *Chlorella* spp. UKM 2 and *C. vulgaris*. *C. sorokiniana* UKM 3 shows that they were able to capture CO₂ at the highest CO₂ concentration (15%) for both integration with the supernatant of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 at 273 mgL⁻¹d⁻¹ and 216 mgL⁻¹d⁻¹, respectively. Meanwhile, the highest CO₂ concentration (15%) reduced the effectiveness of CO₂ capture for *Chlorella* spp. UKM 2 and *C. vulgaris*. It was observed that *C. vulgaris* shows the highest CO₂ capture rate (335 mgL⁻¹d⁻¹) at 5% CO₂ concentration. However, the rate of CO₂ capture by *C. vulgaris* reduced (135 mgL⁻¹d⁻¹) at

15% CO₂ concentration. The results was also due to CO₂ dissolution into media which resulted in low pH of the culture medium that would disrupt the activity of ribulose 1.5-bisphosphate carboxylase-oxygenase, a key enzyme of photosynthesis that can cause inhibition to microalgae growth (Tang et al. 2011). CO₂ dissolution in the media resulted in lower pH due to carbonic acid formation (Mohd Yasin et al. 2015). *Chlorella* sp. was reported able to tolerate CO₂ concentration and fixation rate up to 25% and 25.67 gL⁻¹d⁻¹ (Singh et al. 2018). *Chlorella* sp. was testified most likely to grow in autotrophic regime. It was proved by the literature when maximum specific growth rate of *Chlorella* sp. was double in the media bubbles with CO₂ compared to the media supplemented with NaHCO₃ as the carbon source (Nayak et al. 2017). Therefore, in this study, three different *Chlorella* species were used for CO₂ biofixation.

Table 4 shows COD and nutrients removal by microalgae after POME treatment and CO₂ sequestration study. After POME treatment, it can be observed that COD can be removed in the range of 43-59% with an independent culture of both microalgae species. The COD reduction percentage increase compared to the previous study (29%) for POME treatment by *Chlamydomonas* sp. UKM 6 due to different quality of POME used in different batch operation (Ding et al. 2016). Meanwhile, it was shown that COD removal achieved the highest rate (59%) by inoculation of 5% (v/v) *Chlorella* spp. UKM 8 in POME. It was also found that the inoculum concentration of 5% (v/v), 10% (v/v) and 15% (v/v) does not give significant impact on COD removal from *Chlorella* spp. UKM 8. The other study proved that *C. vulgaris* shows 50.3% of COD removal in POME (Kamarudin et al. 2013) which is in the range of COD reduction by *Chlorella* spp. UKM 8 as indicated in this study (43-59%). Microalgae require essential nutrients such as nitrogen and phosphorus for

TABLE 3. CO₂ capture rate (R_{CO₂} in mgL⁻¹d⁻¹) by UKM 2, UKM 3 and *C. vulgaris* grown in different growth media at different CO₂ concentration

% CO ₂ in mix gas	Supernatant of <i>Chlamydomonas</i> sp. UKM 6			Supernatant of <i>Chlorella</i> spp. UKM 8		
	UKM 2	UKM 3	<i>C. vulgaris</i>	UKM 2	UKM 3	<i>C. vulgaris</i>
5	169	141	335	94	134	246
10	189	201	179	213	167	216
15	66	273	135	118	216	57

TABLE 4. COD and nutrients removal after POME treatment and CO₂ sequestration by microalgae

POME treatment							
Microalgae		<i>Chlamydomonas</i> sp. UKM 6			<i>Chlorella</i> spp. UKM 8		
Inoculum size (v/v)		5%	10%	15%	5%	10%	15%
COD removal (%)		52	56	48	59	43	53
TN removal (%)		56	65	57	43	53	58
TP removal (%)		12	26	16	13	34	19
CO ₂ sequestration							
CO ₂	Contaminant removal (%)	Supernatant of <i>Chlamydomonas</i> sp. UKM 6			Supernatant of <i>Chlorella</i> spp. UKM 8		
		UKM 2	UKM 3	<i>C. vulgaris</i>	UKM 2	UKM 3	<i>C. vulgaris</i>
5%	COD	29	23	36	43	39	49
	TP	19	19	14	31	19	36
10%	COD	30	14	1	30	33	20
	TP	7	12	2	20	13	4
15%	COD	7	7	2	10	13	9
	TP	1	4	16	25	17	2

their growth. Therefore, total nitrogen (TN) and total phosphorus (TP) shows the removal rate in the range of 43-65% and 12-34%, respectively, by *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8. It was shown that *Chlamydomonas* sp. UKM 6 assimilate nitrogen for its growth with highest removal of TN (65%) corresponded to its higher specific growth rate, μ_{max} at 10% (v/v) inoculum size. Ding et al. (2016) reported that *Chlamydomonas* sp. UKM 6 was able to reduce TN in the range of 44-73%. The results for *Chlorella* spp. UKM 8 shows that the increased in inoculum sizes from 5%, 10% and 15% (v/v) slightly increased in TN reduction from 43%, 53% and 57%, respectively. The higher the inoculum size, the lower the percentage of POME being fed as cultivation media (5%, 10%, 15% inoculum: 95%, 90%, 85% POME). Therefore, TN reduction by *Chlorella* spp. was speed up with low supplementation of POME as nutrient sources. The results were in agreement with the research conducted by Khalid et al. (2018) where low concentration of POME supplemented contribute to higher nutrient reduction by microalgae. In addition, previous study also reported that *Chlamydomonas* sp. UKM 6 and *C. vulgaris* able to removed TP up to 69% and 84%, respectively (Ding et al. 2016; Kamarudin et al. 2013) which is higher than the results obtained in this study. Different POME quality and culture condition influenced in nutrients removal. However, all microalgae shows the positive effects in bioremediation of POME in this study. As indicated in Table 4, the first phase of experiment (phycoremediation of POME by *Chlamydomonas* sp. and *Chlorella* spp. UKM 8) only reduced COD, TN and TP of POME up to 59%, 58% and 34%, respectively. Consequently, the remaining nutrients in POME which is not used up in the first stage of POME treatment need to be utilized in the integrated system followed by second stage of CO₂ biofixation.

Meanwhile, the results for CO₂ sequestration study shows that at 5% CO₂ concentration, *C. vulgaris* have the highest COD removal percentage of 36% and 49% when grown in both supernatant of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8, respectively. It was also supported by other study that *C. vulgaris* was tolerant to harsh environmental condition and able to remove COD values effectively (El-Kassas & Mohamed 2014; Kamarudin et al. 2013). It was also observed that TP removal efficiency ranged from 1% to 36% in the media with different CO₂ concentration. The same trends were observed when the TP removal was reduced as CO₂ concentration increased. Increased in CO₂ supplementation reduced the productivity of microalgae, thus inhibiting phycoremediation efficiency and the biomass production (Ebrahimian et al. 2014).

Microalgae biomass contains essential pigments and other component which might be useful for various industrial applications. In this study, microalgae biomass that were harvested after POME treatment and CO₂ sequestration. The microalgae biomass were characterized for its pigment (chlorophyll a and carotenoid) and lipid content. Chlorophyll a and carotenoid are the important elements that indicates the photosynthetic level in microalgae. These pigments were commercially useful in medical field and as food coloring (Cardoso et al. 2017). Table 5 shows the chlorophyll a and carotenoid content and its increment percentage in microalgae biomass after POME treatment and CO₂ sequestration. In microalgae biomass after POME treatment, it was shown that inoculation of 10% (v/v) *Chlamydomonas* sp. UKM 6 in POME accumulated the highest chlorophyll a (11.9 mgL⁻¹) and carotenoid (0.024 mgL⁻¹). These might be due to the effectiveness of *Chlamydomonas* sp. UKM 6 in capturing light due faster lag phase (0.9 day) compared to *Chlorella* spp. UKM 8 (1.3 day) (Table 2). Meanwhile,

TABLE 5. Chlorophyll a and carotenoid content in microalgae biomass before and after POME treatment and CO₂ sequestration

		POME treatment						
Composition (mgL ⁻¹)	Day	<i>Chlamydomonas</i> sp. UKM 6			<i>Chlorella</i> spp. UKM 8			
		Inoculum sizes (v/v)						
		5%	10%	15%	5%	10%	15%	
Chlorophyll a	0	0.5	0.5	0.7	0.1	0.2	0.3	
	21	5.6	11.9	6.6	1.4	1.6	1.5	
	%	92	96	89	91	89	82	
	increment							
Carotenoid	0	0.003	0.003	0.004	0.001	0.001	0.001	
	21	0.022	0.024	0.034	0.005	0.009	0.007	
	%	86	87	89	88	90	86	
	increment							
CO ₂ sequestration								
CO ₂ (%)	Composition (mgL ⁻¹)	Day	Supernatant of <i>Chlamydomonas</i> sp. UKM 6			Supernatant of <i>Chlorella</i> spp. UKM 8		
			UKM 2	UKM 3	<i>C. vulgaris</i>	UKM 2	UKM 3	<i>C. vulgaris</i>
5	Chlorophyll a	0	0.1	0.2	0.5	0.5	1.1	0.4
		10	5.1	6.1	6.2	6.1	6.1	13.0
		%	98	97	92	92	82	97
	increment							
	Carotenoid	0	0.001	0.001	0.001	0.003	0.003	0.002
		10	0.016	0.018	0.018	0.019	0.017	0.033
%		94	94	94	84	82	94	
increment								
10	Chlorophyll a	0	0.1	0.2	0.2	0.3	0.2	0.4
		10	4.2	3.2	5.8	1.2	2.3	1.5
		%	98	94	97	75	91	73
	increment							
	Carotenoid	0	0.001	0.001	0.001	0.001	0.001	0.001
		10	0.002	0.003	0.002	0.003	0.004	0.003
%		50	67	50	67	75	67	
increment								
15	Chlorophyll a	0	0.3	0.7	0.7	0.8	1.0	0.9
		10	0.5	1.3	0.8	0.8	1.1	1.6
		%	40	46	13	0	9	44
	increment							
	Carotenoid	0	0.001	0.002	0.002	0.002	0.002	0.003
		10	0.003	0.004	0.004	0.003	0.003	0.003
%		67	50	50	33	33	0	
increment								

for CO₂ sequestration study, the pattern of chlorophyll a and carotenoid content for each species decreased as CO₂ concentration increased. However, it was observed the total amount of these pigments increased after fermentation shows that the microalgae grew well in the supernatant of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 indicates the feasibility of integrated system between POME treatment and CO₂ sequestration by microalgae.

Among other feedstock for biodiesel production, microalgae are the valued candidate because of its

characteristics which could accumulate high lipid content in a short period of time. Lipid content in biomass act as energy storage in microalgae (Suali & Sarbatly 2012). As shown in Table 6, *Chlamydomonas* sp. UKM6 and *Chlorella* spp. UKM 8 shows the highest lipid content at 10% (v/v) inoculum size which are 12 and 19 mgL⁻¹. The results were corresponded with the highest growth rate of *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 at 10% (v/v) inoculum size which encouraged the highest lipid storage in the biomass. Meanwhile, in CO₂ sequestration study, it was shown that *C. vulgaris* had the

highest lipid content as the CO₂ concentration increased (up to 27 mgL⁻¹). It was demonstrated by other studies that microalgae stored lipid in the excess amount of CO₂ due to the additional of carbon uptake by the cells that

initiates lipid accumulation in microalgae (Lam & Lee 2013). Therefore, it can be observed that the integrated system of POME treatment and CO₂ sequestration also contribute to the production of valuable microalgae

TABLE 6. Lipid content in microalgae biomass after POME treatment and CO₂ sequestration

Microalgae		POME treatment					
		<i>Chlamydomonas</i> sp. UKM 6			<i>Chlorella</i> spp. UKM 8		
		Inoculum size (v/v)					
		5%	10%	15%	5%	10%	15%
Lipid content (mg/g)		10.3	11.1	6.4	9.8	17.9	3.2
CO ₂ (%)		CO ₂ sequestration					
		Supernatant of <i>Chlamydomonas</i> sp. UKM 6			Supernatant of <i>Chlorella</i> spp. UKM 8		
		UKM 2	UKM 3	<i>C. vulgaris</i>	UKM 2	UKM 3	<i>C. vulgaris</i>
5	Lipid content (mg/g)	24.1	23.2	19.6	28.6	12.9	16.2
10		25.4	24.1	20.0	12.0	29.2	38.3
15		47.5	29.2	37.7	25.0	29.1	90.0

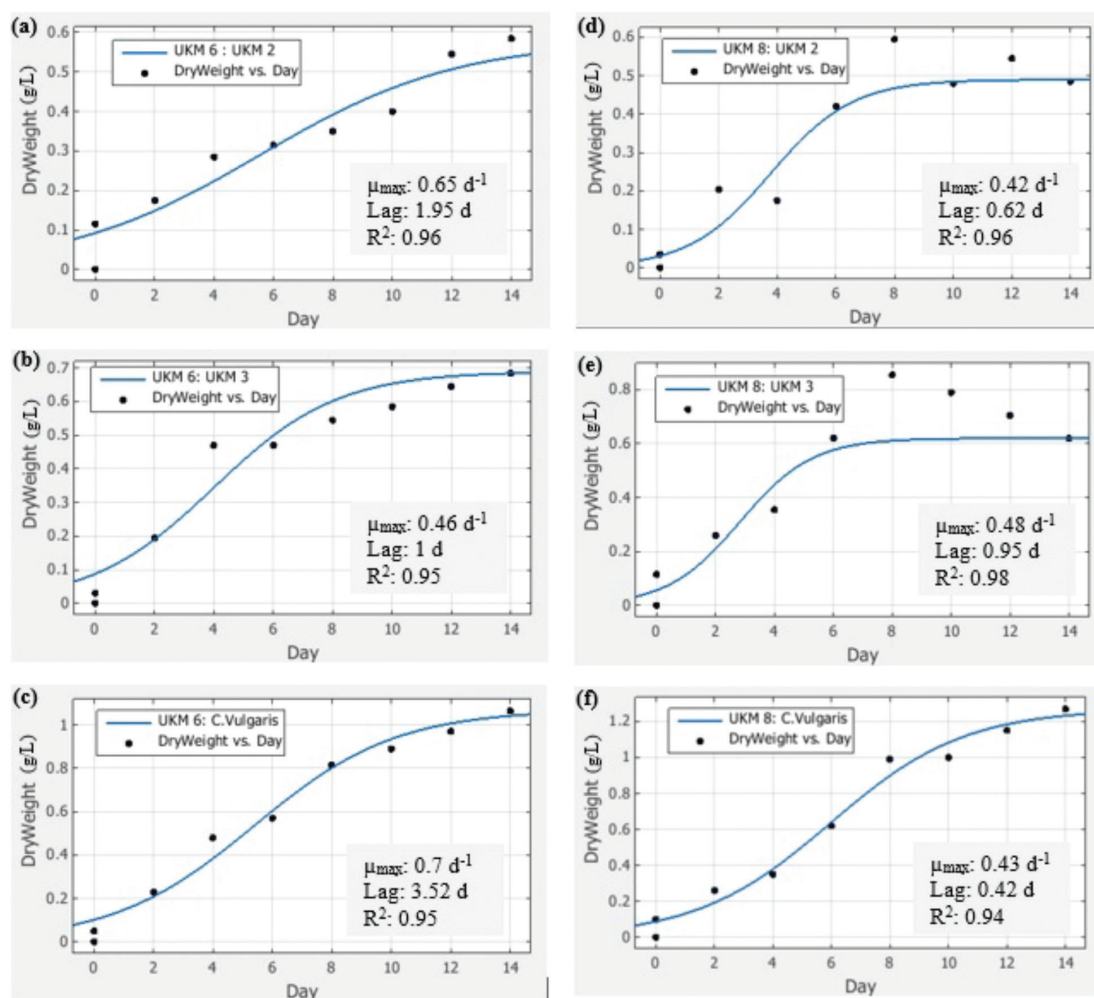


FIGURE 2. Growth profile and kinetic parameters of microalgae (a) UKM 2, (b) UKM 3 and (c) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 6 and (d) UKM 2, (e) UKM 3 and (f) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 8 supplied with 5% CO₂ in mix gas

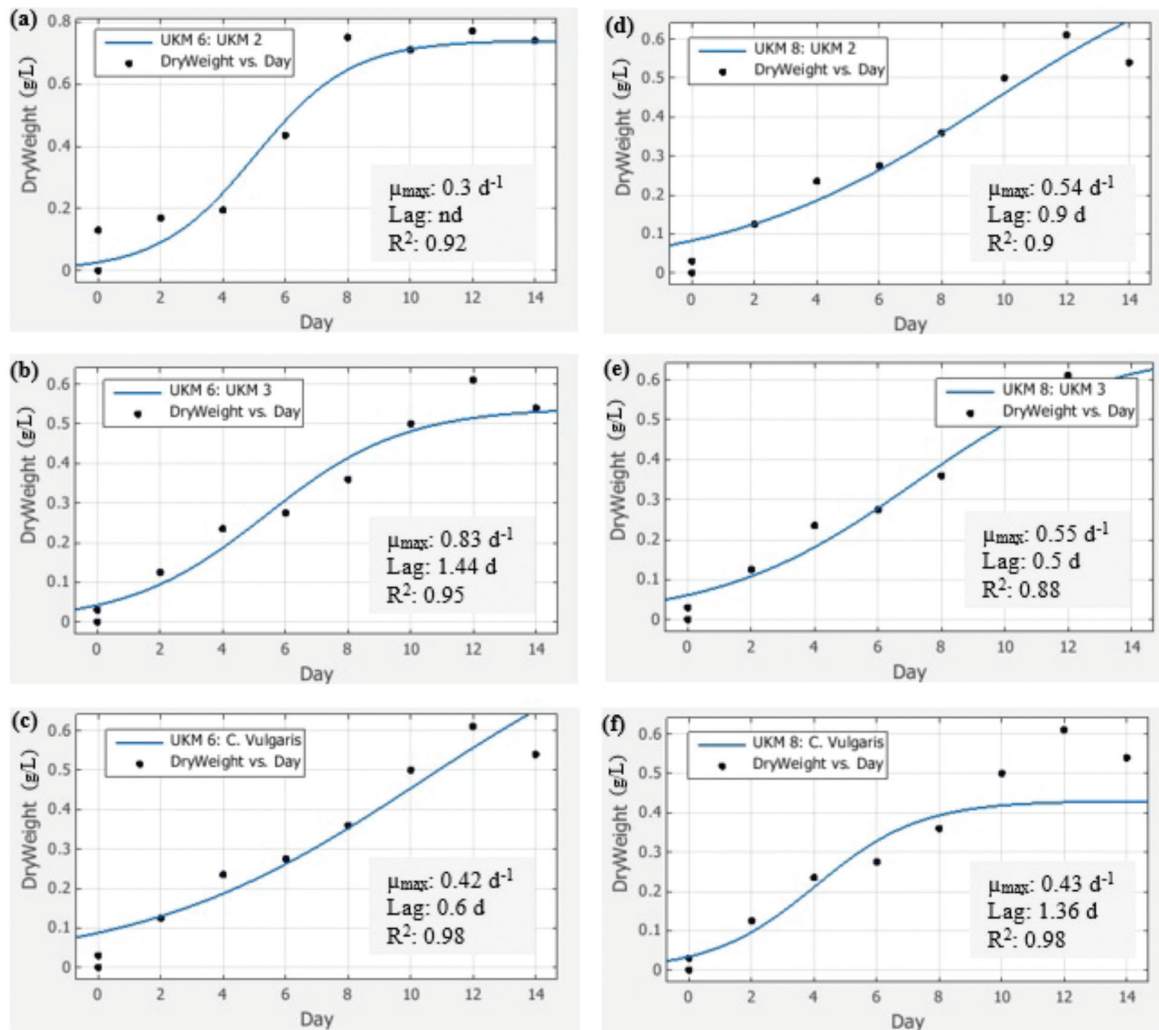


FIGURE 3. Growth profile and kinetic parameters of microalgae (a) UKM 2, (b) UKM 3 and (c) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 6 and (d) UKM 2, (e) UKM 3 and (f) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 8 supplied with 10% CO₂ in mix gas

biomass. The potential production of biodiesel from microalgae biomass do not compete with oil crops and food which makes the overall system much more valuable (Zainal et al. 2012).

The feasibility of integrated system of POME treatment and CO₂ sequestration were shown in this study. Both system were able to phycoremediate excess contaminant and nutrients available in POME. In addition, all microalgae were able to sequester CO₂ for their growth. Throughout this study, harvested microalgae biomass derived after POME treatment and CO₂ sequestration contains significant amount of chlorophyll a, carotenoid and lipids which have the potential for high value products in industrial scale.

CONCLUSION

A novel native green microalgae species have the potential to grow in POME and to remove nutrients

efficiently. Treated POME can be integrated with CO₂ sequestration and at the same time phycoremediate and utilize sufficient nutrients from POME. Microalgae biomass that phycoremediate POME and CO₂ contains significant amount of chlorophyll a, carotenoid and lipid which then can be the potential sources for high-value products. This study showed that the integrated system of POME treatment and CO₂ sequestration by microalgae were feasible for the future application in palm oil industries. The strategy of POME utilization should be studied further in order to optimize the biomass growth and nutrients removal.

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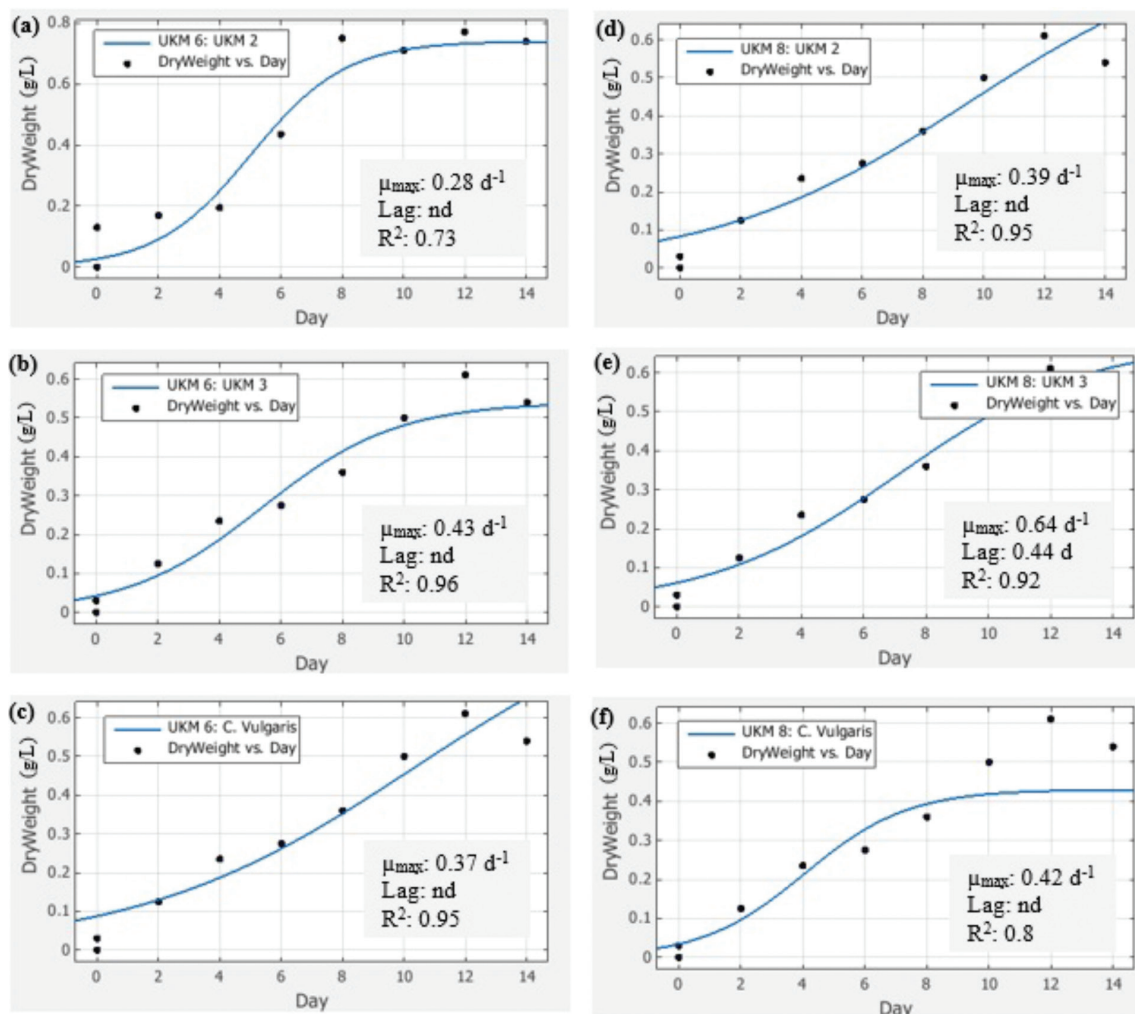


FIGURE 4. Growth profile and kinetic parameters of microalgae (a) UKM 2, (b) UKM 3 and (c) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 6 and (d) UKM 2, (e) UKM 3 and (f) *C. vulgaris* grown in supernatant after POME treatment treated with UKM 8 supplied with 10% CO₂ in mix gas

REFERENCES

- Ahmad, A. & Krimly, M.Z. 2014. Palm oil mill effluent treatment process evaluation and fate of priority components in an open and closed digestion system. *Current World Environment* 9: 2.
- Amin, I. 2014. *Malaysian Palm Oil Industry*. Malaysian Palm Oil Council (MPOC). http://www.mpoc.org.my/Malaysian_Palm_Oil_Industry.aspx. Accessed on 31 January 2018.
- Babu, A., Katam, K., Gundupalli, M.P. & Bhattacharyya, D. 2017. Nutrient removal from wastewater using microalgae: A kinetic evaluation and lipid analysis. *Water Environment Research* 90(6): 520-529.
- Bligh, E.G. & Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917.
- Cardoso, L.A.C., Karp, S.G., Vendruscolo, F., Kanno, K.Y.F., Zoz, L.I.C. & Carvalho, J.C. 2017. Biotechnological production of carotenoids and their applications in food and pharmaceutical products. *Carotenoids*. InTech Open.
- Demirbaş, A. 2008. Production of biodiesel from algae oils. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* 31(2): 163-168.
- Department of Environment. 1977. *Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations*. Department of Environment. Second Schedule (Regulation 12(2) and (3)) Parameter Limits for Watercourse Discharge.
- Ding, G.T., Yaakob, Z., Takriff, M.S., Salihon, J. & Abd Rahaman, M.S. 2016. Biomass production and nutrients removal by a newly-isolated microalgal strain *Chlamydomonas* sp. in palm oil mill effluent (POME). *International Journal of Hydrogen Energy* 41(8): 4888-4895.
- Ebrahimian, A., Kariminia, H.R. & Vosoughi, M. 2014. Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater. *Renewable Energy* 71: 502-508.
- El-Kassas, H.Y. & Mohamed, L.A. 2014. Bioremediation of the textile waste effluent by *Chlorella vulgaris*. *The Egyptian Journal of Aquatic Research* 40(3): 301-308.
- Environmental Quality (Clean Air) Regulations. 1978 PU(A) 280/1978.
- Holdmann, C., Schmid-Staiger, U., Hornstein, H. & Hirth, T. 2018. Keeping the light energy constant - Cultivation of *Chlorella sorokiniana* at different specific light availabilities and different photoperiods. *Algal Research* 29: 61-70.

- Kamarudin, K.F., Yaakob, Z., Rajkumar, R., Takriff, M.S. & Tasirin, S.M. 2013. Bioremediation of palm oil mill effluents (POME) using *Scenedesmus dimorphus* and *Chlorella vulgaris*. *Advanced Science Letters* 19(10): 2914-2918.
- Khalid, A.A.H., Yaakob, Z., Abdullah, S.R.S. & Takriff, M.S. 2018. Growth improvement and metabolic profiling of native and commercial *Chlorella sorokiniana* strains acclimatized in recycled agricultural wastewater. *Bioresource Technology* 247: 930-939.
- Lam, M.K. & Lee, K.T. 2013. Effect of carbon source towards the growth of *Chlorella vulgaris* for CO₂ bio-mitigation and biodiesel production. *International Journal of Greenhouse Gas Control* 14: 169-176.
- Mohd Yasin, N.H., Maeda, T., Hu, A., Yu, C.P. & Wood, T.K. 2015. CO₂ sequestration by methanogens in activated sludge for methane production. *Applied Energy* 142: 426-434.
- Nayak, M., Suh, W.I., Lee, B. & Chang, Y.K. 2017. Enhanced carbon utilization efficiency and FAME production of *Chlorella* sp. HS₂ through combined supplementation of bicarbonate and carbon dioxide. *Energy Conversion and Management* 154: 45-52.
- Nur, M.M.A., Setyoningrum, T.M. & Budiaman, I.G.S. 2018. Potency of *Botryococcus braunii* cultivated on palm oil mill effluent (POME) wastewater as the source of biofuel. *Environmental Engineering Research* 22(4): 1-8.
- Rao, C.S., Sathish, T., Brahmaiah, P., Kumar, T.P. & Prakasham, R.S. 2009. Development of a mathematical model for *Bacillus circulans* growth and alkaline protease production kinetics. *Journal of Chemical Technology and Biotechnology* 84(2): 302-307.
- Sforza, E., Gris, B., Silva, C.E.D.F., Morosinotto, T. & Bertucco, A. 2014. Effects of light on cultivation of *Scenedesmus obliquus* in batch and continuous flat plate photobioreactor. *Chemical Engineering Transactions* 38: 211-216.
- Singh, P., Singh, R.K. & Kumar, D. 2018. Microalgae: Potential agents for carbon dioxide mitigation. In *Microbes for Climate Resilient Agriculture*, edited by Kashyap, P.L., Srivastava, A.K., Tiwari, S.P. & Kumar, S. New York: John Wiley & Sons. p. 64.
- Singh, R.P., Ibrahim, M.H., Esa, N. & Iliyana, M.S. 2010. Composting of waste from palm oil mill: A sustainable waste management practice. *Reviews in Environmental Science and Biotechnology* 9(4): 331-344.
- Suali, E. & Sarbatly, R. 2012. Conversion of microalgae to biofuel. *Renewable and Sustainable Energy Reviews* 16(6): 4316-4342.
- Tang, D., Han, W., Li, P., Miao, X. & Zhong, J. 2011. CO₂ biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO₂ levels. *Bioresource Technology* 102(3): 3071-3076.
- Watada, A.E., Norris, K.H., Worthington, J.T. & Massie, D.R. 1976. Estimation of chlorophyll and carotenoid contents of whole tomato by light absorbance technique. *Journal of Food Science* 41(2): 329-332.
- Wu, T.Y., Mohammad, A.W., Jahim, J.M. & 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.
- Zainal, A., Yaakob, Z., Takriff, M.S., Renganathan, R. & Ghani, J.A. 2012. Phycoremediation in anaerobically digested palm oil mill effluent using Cyanobacterium, *Spirulina platensis*. *Journal of Biobased Materials and Bioenergy* 6: 704-709.
- Zheng, H., Gao, Z., Yin, F., Ji, X. & Huang, H. 2012. Effect of CO₂ supply conditions on lipid production of *Chlorella vulgaris* from enzymatic hydrolysates of lipid-extracted microalgal biomass residues. *Bioresource Technology* 126: 24-30.

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