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The potential of biodiesel production from *Botryococcus* sp. biomass after phycoremediation of domestic and industrial wastewater

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Abstract. The aim of the present work is to investigate the capability of microalgae, known as *Botryococcus* sp. for wastewater phycoremediation and potential biodiesel production. The vertical closed photobioreactors (PBR) were employed and supplemented with domestic wastewater (DW) and food industry wastewater (FW) at different batch of study. The cultivation was conducted under natural outdoor condition for 12 days. The results revealed that the removal of pollutant and nutrients presence in both wastewaters with constantly decrease proportionate to the increase in cultivation time. The chemical oxygen demand (COD), total phosphorus (TP) and total organic carbon (TOC) were successfully removed up to 84.9%, 69.3% and 93.3%, respectively in DW while 96.1%, 35.5% and 87.2%, respectively in FW. The result on FT-IR analysis of microalgae oil was shown comparable with conventional palm oil based biodiesel in term of IR spectra. This study suggests that *Botryococcus* sp. has tremendous potential in pollutants removal and biodiesel production for renewable energy development.

1. Introduction

By 2050, global population are predicted requiring more supply of food, fuel and water between 50% and 70% [1]. This is due to the rapid growth of people over the world. Hence, the environmental pollution caused by untreated wastewater and excessive carbon dioxide emissions due to the burning of hydrocarbon from petrol and diesel engine has become a popular debate among researcher [2, 3]. In fact, the world is now experiencing a shortage of conventional petroleum resources and has promoted the development of new ideas in the renewable energy technologies [4, 5].

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The most promising approach is via microalgae cultivation using wastewater-coupled with biodiesel production [6, 7]. The superiority of applying microalgae as a source of crude oil for biodiesel production are high in biomass and lipid productivity compared to terrestrial crops and effectively capture CO_2 from the atmospheric [8–10]. However, biodiesel production from microalgae is relatively costing if the sustainable perspectives not fully given attention [11]. Therefore, the application of wastewater as a growth media indirectly reduce the cost to replace the synthetic media used in cultivation since most of the wastewater readily contained important nutrients for their growth. At the same time, pollutants including a very harmful metal elements presence in wastewater were able to be removed significantly in a sustainable way [7, 12–15].

Wastewater bioremediation using microalgae cultivation known as phycoremediation offers a lot of advantages compared to conventional method. As intensively discussed in our review article [16], phycoremediation is a cost-effective, eco-friendly and a safe process besides microalgae used are non-pathogenic photosynthesis organisms then they do not produce any toxic substance. Other than that, phycoremediation effectively removes excessive nutrients in wastewater thereby reducing total dissolved solid and minimise the sludge formation to an enormous extent. Also, microalgae are great in CO_2 absorption from the air thereby contributing to the reduction of greenhouse gases. Interestingly, microalgae cultivation in wastewater provides a significant potential for renewable energy production such as biodiesel, biogas and bio-methane generation from their biomass [17–19].

The previous researcher has reported various microalgae species belonging to *Chlorella vulgaris*, *Scenedesmus obliquus*, *Desmodesmus* sp., *Micratinium reisseri* and *Chlorococcum* sp. to effectively reduce the contaminations such as nitrogen, phosphorus, metals and inorganic carbon in different wastewater along with biomass production for biodiesel production [1, 15, 20–22]. So far, however, there has been little discussion about microalgae *Botryococcus* sp. cultivation in domestic and industrial wastewater for potential biodiesel production. Previously, *Botryococcus braunii* was cultivated in piggery wastewater for hydrocarbon production combined with nutrients removal [23]. In 2013, Can et al. [24] used *Botryococcus braunii* to treat domestic wastewater along with total lipid determination under indoor condition. Meanwhile, Raj GP et al. [25] applied *Botryococcus braunii* as a phycoremediation tools for domestic wastewater collected in different site around the Chennai city, India. Therefore, it is essential to conduct a comprehensive study about microalgae *Botryococcus* sp. cultivated in wastewater for potential biodiesel production. Accordingly, this study aims to experimentally investigate the cultivation of *Botryococcus* sp. locally isolated for wastewater bioremediation along with potential biodiesel production by using FT-IR analysis.

2. Materials and method

2.1. Microalgae preparation and wastewaters sampling

The freshwater green microalgae, *Botryococcus* sp. employed in this research was obtained from tropical rainforest located in the southern region of Peninsular Malaysia [26]. The collected microalgae were through the isolation process then purified and morphologically identified according to structure, colour, shape, and pattern prior to molecular identification [26, 27]. The preparation of microalgae *Botryococcus* sp. inoculum follows Gani et al. [26]. The cell density was counted using the hemocytometer [29] at an initial cell density of 1×10^6 cell/mL and then algae cells were transferred to each photobioreactor. This microalgae cell concentration according to a suggestion from our previous study in which 1×10^6 cell/mL was the best concentration to be inoculated in wastewater in term of biomass productivity [26].

The domestic wastewater (DW) was collected from wastewater treatment plant located on the main campus of Universiti Tun Hussein Onn Malaysia, Johor. This plant receives daily wastewater generated from the whole campus area. Meanwhile, the food industry wastewater (FW) was obtained from food manufacturing industries located in Rengit, Johor. Basically, the core business of this industry is processing and the production of snack foods which is supplied in the surrounding area of

Batu Pahat. The samples were collected using acid washed sample bottles at the site and immediately transferred to the laboratory and preserved at temperatures below 4°C in a refrigerator [29]. The chemical oxygen demand (COD), total phosphorus (TP) and total organic carbon (TOC) had been tested using DR 6000 Spectrophotometer (Hach, USA) according to Method 8000, Method 10127 and Method 10129, respectively. The wastewater samples were filtered using a membrane filter (Whatman) with a 0.45µm pore size to remove other microorganisms and suspended solids before commencing the experiments.

2.2. Batch cultures in closed photobioreactors

Four closed photobioreactors constructed with a total capacity of 25L each was used. The cultivation of *Botryococcus* sp. had been conducted in a different batch of wastewater treatment which is domestic wastewater (DW) and food processing wastewater (FW). A total of 22L of prepared and filtered wastewater has been transferred to each three photobioreactors while left one of photobioreactor was filled up with distil-water as a control sample. The cultures were continuously aerated using a mini air pump (maximum output: 3L/min) from the bottom of photobioreactors to ensure microalgae homogenised in wastewater. Both batches of cultures were carried out under an outdoor condition with 24°C - 33°C of temperature and natural sunlight (200 lux – 18000 lux) for 12 days [30]. The quality of wastewater parameter was tested at an interval of 3 days and total removal efficiencies were calculated using Equation 1.

$$Removal,\ \% = \frac{Initial\ \text{conc.}\ (mg.L^{-1})\ \text{- Final conc.}\ (mg.L^{-1})}{Initial\ \text{conc.}\ (mg.L^{-1})} \times 100 \quad (1)$$

2.3. Harvesting of microalgae biomass

Flocculation technique using aluminium sulfate, $Al_2(SO_4)_3$ as an inorganic coagulant was used to harvest the *Botryococcus* sp. biomass from both wastewaters. The selection of this technique due to the low-cost and high efficiency in microalgae harvesting as comprehensively discussed by Vandamme et al. [31]. Flocculation steps and procedures were done according to laboratory jar test standard method [32]. An optimum amount of alum about 177.74 mg/L with 99.3% of efficiency was used to harvest microalgae grew in DW. Meanwhile, about 166 mg/L of alum dose with 92.4% of harvesting efficiency was employed to recover microalgae cultivated in FW. Both optimum value of flocculation were obtained from our optimisation study using response surface methodology (data not shown). After that, concentrated biomass was dried at 60°C for 24 hours using a universal drying oven [33].

2.4. Microalgae oil extraction and analysis

The extraction of crude oil from microalgae biomass was conducted according to the EPA Method 9071B (n-Hexane extractable material) using soxhlet apparatus. The sample preparation follows Gani et al. [33]. Fourier Transform Infra-Red (FT-IR) Spectrometer of Thermo-Scientific (Nicole 6700) was used to analyse the extracted bio-oil samples. The FT-IR spectrophotometer has been supplemented with an accessory called Attenuated Total Reflectance (ATR) to enhance the use and application of the instrument in a way that materials like filmy/papery; liquid nature properties analysis. The Zinc Selenium (ZnSe) pellets are taken for correction of background spectrum. The FT-IR spectra are recorded over a range of wave number from 4000 to 600 cm^{-1}. The transmittance peaks were analysed according to principal IR absorption for certain functional groups. Each sample was analysed in triplicate [34].

2.5. Statistical analysis

All experiments were conducted in triplicates for each culture. Data analysis of average, mean differences, standard deviation, and the graph for each experiment were done using packaging software tool OriginPro version 2015.

3. Results and discussion

3.1. Wastewater parameter removal analysis

The removal of chemical oxygen demand (COD), total phosphorus (TP) and total organic carbon (TOC) from domestic wastewater (DW) and food industry wastewater (FW) by microalgae, *Botryococcus* sp. cultivation as a function of phycoremediation time are illustrated in Figure 1.

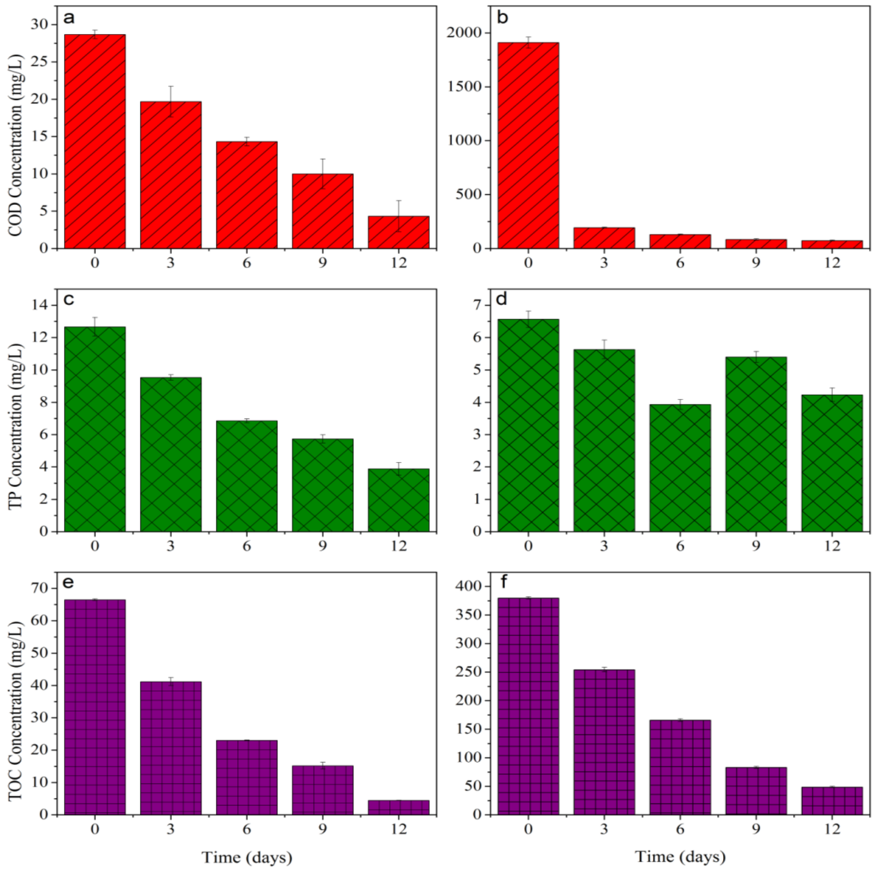

Figure 1. The reduction of COD, TP and TOC during phycoremediation for domestic wastewater (a, c and e) and food processing wastewater (b, d and f)

According to Figure 1 (a) and (b), the initial COD concentration of FW (1910 mg/L) is extremely higher than DW (28.7mg/L). The trend reduction of COD from DW was steadily decreased with the treatment time. Meanwhile, the reduction of COD from FW was dramatically going down after 3 days of phycoremediation then almost maintained until the day 6 and finally reduced up to 73.7 mg/L on the Day 12. Therefore, the COD content in DW was reduced from 28.7 mg/L to 4.33 mg/L which is equivalent to 84.9% of removal efficiency (Figure 2). However, COD concentration in FW was reduced from 1910 mg/L to 73.7 mg/L at the end of treatment in which significantly contribute about

96.1% of removal efficiency (Figure 2). The present findings also support our previous preliminary study which found that *Botryococcus* sp. was effectively remediate COD presence in FW up to 70.7% when we used 1×10^3 cell/mL of algae concentration in the treatment [4]. On the other study, green microalgae, *Chlorella vulgaris* reduced COD in domestic wastewater mixed with seawater ranged from 83.6% to 90.8% while *Chlorella salina* removed between 87.3% and 90.97% [7]. Hadiyanto et al. [35] reported that accumulation of COD by microalgae cultivation because of the absorption of carbon dioxide since microscopic algae effectively convert COD concentration into their carbon sources of living. In fact, the chemical reaction of carbon in organic nutrients releasing carbon dioxide also contributes to the reduction of COD content in wastewater [7].

The data in Figure 1 (c) and (d), showed the application of *Botryococcus* sp. in DW and FW treatment reduced the TP contents during 12 days of phycoremediation. The highest removal of TP in DW and FW was 69.3% and 35.5%, respectively. With that, TP concentration in DW successfully reduced from 12.7 mg/L to 3.89 mg/L while 6.6 mg/L to 4.23 mg/L for FW after 12 days (Figure 2). These results were in line with findings obtained by another previous study. Arora et al. [6] found that the same green microalgae but different species called *Chlamydomonas debaryana* was successfully reduced TP about 80.6% in DW, 78.6% in dairy water, 71.8% in sewage water and 65% in paper wastewater. They stated that sudden decrease can be described by growth activity, nutrients removal and accumulation of phosphorus due to high pH value during phycoremediation [4, 6, 7]. Besides that, phosphorus assimilation by microalgae is stored and accumulated in the algae cells as polyphosphate granules, magnesium and potassium [7].

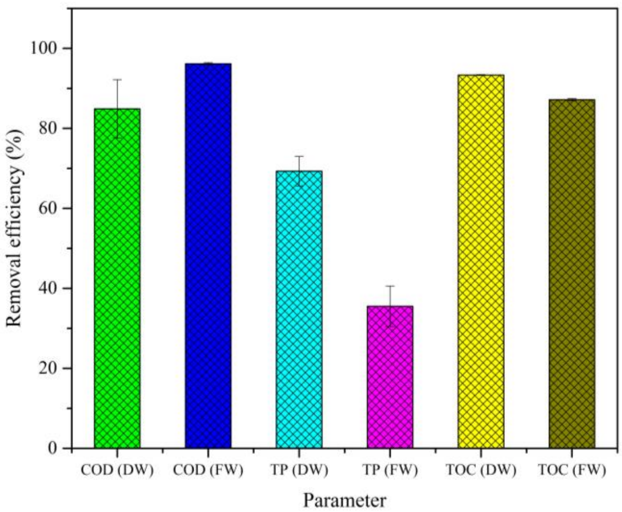

Figure 2. Removal efficiency of COD, TP and TOC from domestic wastewater (DW) and food processing wastewater (FW)

The total removal efficiencies of TOC in DW and FW also determined at the interval during treatment using *Botryococcus* sp. (Figure 1). Similarly, TOC removal also experienced the same pattern for both wastewaters. The maximal TOC removal from DW and FW was 93.3% and 87.2%, respectively. A drastic decrease in TOC was observed within 12 days for each of the wastewater. Mahapatra et al. [36] revealed that the removal of TOC was 86%, occurred when they employed mixotrophic algae consortia to treat municipal wastewater, which is in good agreement with the results of the present study. However, TOC removal in the present study showed better results compared to our previous study when applied *Botryococcus* sp. in dairy wastewater in which capable to reduce TOC about 65.1% only [13].

3.2 FT-IR analysis

The crude oil extracted from microalgae biomass can be used as a feedstock for biodiesel production [37]. The hydrocarbons are the main elements of petrodiesel and similar to the biodiesel properties obtained from microalgae oil [38]. The FT-IR spectra of bio-oil extracted from *Botryococcus* sp. biomass cultivated in different wastewaters (domestic and food processing) are shown in Figure 3. The FT-IR spectra of a triplicate sample of each culture medium generated the same pattern of bands then producing the similar wavenumber (cm^{-1}). Therefore, the bands obtained from crude oil in each culture medium were averaged as illustrated in Figure 3. The possible functional group composition assignments are listed in Table 1.

The strong and broad absorbance peak of O-H stretching was obtained in culture media of DW and FW at 3241.71 cm^{-1} and 3339.45 cm^{-1}, respectively. The presence of this peak normally caused by water or alcohol in the bio-oil [39]. The data shown the absorption peaks are almost the same for all three oil sample (Figure 3). The C-H stretching absorption occurs at the wavenumber between 2850 cm^{-1} and 3000 cm^{-1}. These peaks appear strong in all oil sample tested due the presence of alkanes and alkyls (Table 1). However, alkanes in the form of methyl only exist in algae oil cultivated in both wastewaters because of the medium bending of CH_3. In the range of 1085 cm^{-1} to 1150 cm^{-1}, all oil sample (Figure 3) tested contain ether, epoxides, acetals and ketals due to the strong C-O-C stretching vibration. The FT-IR analysis falls mainly in the very related to the hydrocarbon categories such as fatty acid, fatty acid methyl esters, ketones and aldehydes (Table 1 and Figure 3).

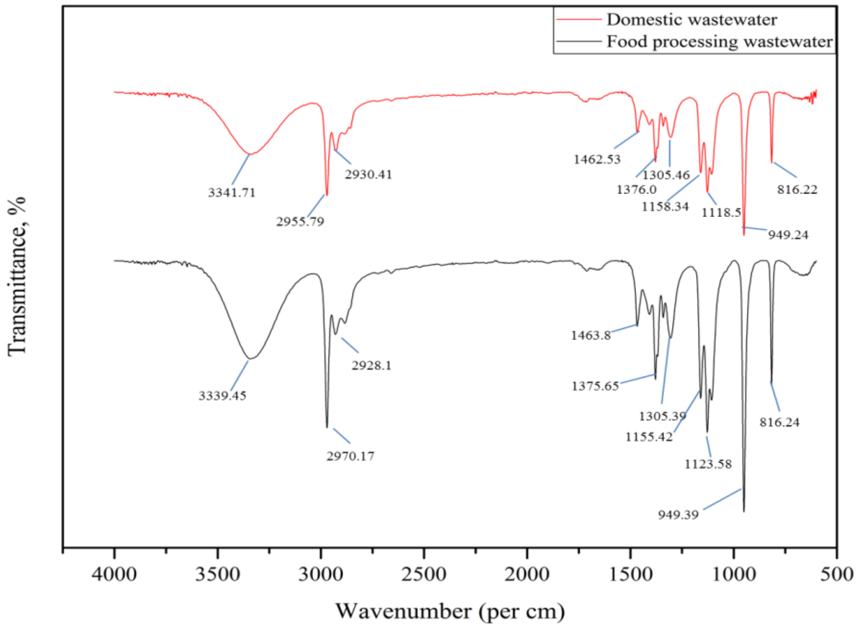

Figure 3. FT-IR spectra analysis of *Botryococcus* sp. oil harvested after phycoremedation of wastewaters.

Similarly, Shuping et al. [40] observed the same categories when they examined bio-oil obtained from *Dunaliella tertiolecta*. This is because of the microalgae biomass composed of proteins, fats and raw cellulose with the total crude protein and crude fat extracted using organic solvent [40]. Interestingly, the data clearly indicates that the spectrum of DW and FW almost in line with palm oil based biodiesel spectrum [41]. The triglycerides and phospholipids do exist in the spectrum of *Botryococcus* sp. which similar to the spectrum of methyl ester in petrodiesel spectra [42]. These results are in good agreement with previous past studies [5, 9, 36, 38, 42].

4. Conclusion

The *Botryococcus* sp. in a vertical closed photobioreactor successfully treated domestic and food industrial wastewater. The microalgae presented efficient removal of chemical oxygen demand (COD), total phosphorus (TP) and total organic carbon (TOC) for both wastewaters. The FT-IR profiles analysis showed the applicability of the derived microalgae oil as a potential valuable for biodiesel production. This eco-friendly approach could potentially eliminate the use of the expensive synthetic medium for biodiesel production since the consolidation method of using wastewater is a more low-cost strategy. Considerably more work will need to be done to conduct the transesterification process after obtained the crude bio-oil to produce standard biodiesel.

Table 1. FT-IR band assignments

Absorption range (cm^{-1})	Absorption (cm^{-1})		Group	Intensity	Class of compound
	DW	FW			
2500-3500	3341.71	3339.45	O-H Stretching	Strong, broad	Alcohols, phenols, water impurities
2850-3000	2955.79, 2930.41	2970.17, 2928.1	C-H Stretching	Strong	Alkanes and alkyls
1735-1750	N/A	N/A	C=O Stretching	Very strong	Esters (Aliphatic)
1450-1470	1462.53	1463.8	C-H Bending	Strong	Alkanes (Methylene)
1370-1390	1376.00	1375.65	CH_3 C-H Bending	Medium	Alkanes (Methyl)
1000-1350	1305.46	1305.39	C-F Stretching	Very strong	Alkyl halides
1200-1275	N/A	N/A	=C-O-C sym. & asym. Stretching	Medium-strong	Ethers
1125-1205	1158.34	1155.42	C-O Stretching	Medium-strong	Alcohols, phenols
1085-1150	1118.50	1123.58	C-O-C stretching	Strong	Ethers, epoxides, acetals, ketals
790-840	816.22	816.24	=C-H Bending	Strong	Alkenes
665-730	N/A	N/A	=C-H Bending	Medium-strong, broad	

*N/A = Not available

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References

[1] Yang I S, Salama E S, Kim J O, Govindwar S P, Kurade M B, Lee M, Roh H S and Jeon B H 2016 *Energy Convers. Manag.* **117** 54–62
[2] Furuhashi K, Hasegawa F, Saga K, Kudou S, Okada S, Kaizu Y, and Imou K 2016 *Biomass and*

Bioenergy **91** 83–90
[3] Safaai N S M, Noor Z Z, Hashim H, Ujang Z, and Talibc J 2011 *Environ. Prog. Sustain. Energy* **30** 658–665
[4] Gani P, Sunar N M, Matias-peralta H, and Abdul Latiff A A 2016 *J. Eng. Appl. Sci.***11** 7288–7292
[5] Mahapatra D M and Ramachandra T V 2013 *Curr. Sci.***105** 47–56
[6] Arora N, Patel A, Sartaj K, Pruthi P A, and Pruthi V 2016 *Environ. Sci. Pollut. Res.*1–11
[7] El-Sheekh M M, Farghl A A, Galal H R, and Bayoumi H S 2015 *Rend. Lincei* 1–10
[8] Wang G and Wang T 2011 *J. Am. Oil Chem. Soc.* **89** 135–143
[9] Prabakaran P and Ravindran A D 2012 *Curr. Sci.* **102** 616–620
[10] Tasić M B, Pinto L F R, Klein B C, Veljković V B, and Filho R M 2016 *Renew. Sustain. Energy Rev.* **64** 260–270
[11] Arnold M 2013 *Sustainable algal biomass products by cultivation in waste water flows* (Finland)
[12] Gani P, Sunar N M, Matias-Peralta H, Abdul Latiff A A, Kamaludin N S, Parjo U K, Emparan Q, and Ming E C 2015 *Appl. Mech. Mater.***773–774** 1312–1317
[13] Gani P, Sunar N M, Matias-Peralta H, Abdul Latiff A A, Joo I T K, Parjo U K, Emparan Q, and Ming E C 2015 *Appl. Mech. Mater.* **773–774** 1318–1323
[14] Razak A R A, Sunar N M, Alias N A, Gani P, and Subramaniam M 2016 *MATEC Web Conf.* **47**
[15] Lu Q, Zhou W, Min M, Ma X, Chandra C, Doan Y T T, Ma Y, Zheng H, Cheng S, Griffith R, Chen P, Chen C, Urriola P E, Shurson G C, Gisler??d H R, and Ruan R 2015 *Bioresour. Technol.* **198** 189–197
[16] Gani P, Sunar N M, Matias-peralta H, Abdul Latiff A A, Parjo U K, and Razak A R A 2015 *Adv. Environ. Biol.* **9** 1–8
[17] Kim J, Yoo G, Lee H, Lim J, Kim K, Kim C W, Park M S, and Yang J W 2013 *Biotechnol. Adv.* **31** 862–76
[18] Prajapati S K, Kaushik P, Malik A, and Vijay V K 2013 *Bioresour. Technol.* **135** 232–8
[19] Tijani H, Abdullah N, and Yuzir A 2015 *Renew. Sustain. Energy Rev.* **52** 1610–1622
[20] Ji F, Liu Y, Hao R, Li G, Zhou Y, and Dong R 2014 *Bioresour. Technol.***161** 200–207
[21] Ji M K, Kabra A N, Salama E S, Roh H S, Kim J R, Lee D S, and Jeon B H 2014 *Bioresour. Technol.* **157C** 84–90
[22] Ramachandra T V, Durga Madhab M,Shilpi S, and Joshi N V 2013 *Renew. Sustain. Energy Rev.* **21** 767–777
[23] An J, Sim S, Lee J S, and Kim B W 2003 *J. Appl. Phycol.* **15** 185–192
[24] Can Ş S, Dem V, Korkmaz Ş A, and Can E 2013 *J. Food, Agric. Environ.* **11** 2128–2130
[25] Raj GP A S, Elumalai, Sangeetha, and Singh R 2015 *J. Bioremediation Biodegrad.* **06**
[26] Andersen R A 2005 *Algal Culturing Techniques* (ELSEVIER Academic Press)
[27] Prescott G W 1978 *How to Know the Freshwater Algae* (USA: DUBUQUE, IOWA)
[28] Gani P, Sunar N M, Matias-Peralta H, Abdul Latiff A A, and Razak A R A 2016 *Appl. Ecol. Environ. Res.***14** 399–409
[29] APHA 2012 *Standard Methods for the Examination of Water and Wastewater: American Public Health Association, AWWA* (American Water Works Association)
[30] Gani P, Sunar N M, Matias-Peralta H, Abdul Latiff A A and Fuzi S F Z M 2016 *Prep. Biochem. Biotechnol.*
[31] Vandamme D, Foubert I, and Muylaert K 2013 *Trends Biotechnol.* **31** 233–239
[32] ASTM 1995 *"Standard Practice for Coagulation-Flocculation Jar Test of Water E1-1994 R. D 2035-80* (Annual Book of ASTM Standards) **11** 2
[33] Gani P, Sunar N M, Matias-peralta H, and Abdul Latiff A A 2016 *J. Eng. Appl. Sci.***11** 7431–7436
[34] Kothari R, Prasad R, Kumar V, and Singh D P 2013 *Bioresour. Technol.* **144** 499–503
[35] Hadiyanto, Christwardana M, and Soetrisnanto D 2013 *J. Environ. Sci. Technol.* **6** 79–90

[36] Mahapatra D M, Chanakya H N, and Ramachandra T V 2014 *Bioresour. Technol.* **168** 142–50
[37] Maity J P, Bundschuh J, Chen C Y, and Bhattacharya P 2014 *Energy*, **78** 104–113
[38] Banerjee A, Sharma R, Chisti Y, and Banerjee U C 2002 *Crit. Rev. Biotechnol.* **22** 245–79
[39] Zou S, Wu Y, Yang M, Li C, and Tong J 2010 *Energy Environ. Sci.* **3** 1073–1078
[40] Shuping Z, Yulong W, Mingde Y, Kaleem I, Chun L, and Tong J 2010 *Energy* **35** 5406–5411
[41] Mumtaz M W, Mukhtar H, Anwar F, and Saari N 2014 *Sci. World J.* **2014** 1–11
[42] Santhoshkumar K, Prasanthkumar S, and Ray J G 2015 *Am. J. Plant Sci.* **6** 2453–2460
[43] Yin N C, Yaakob Z, Ali E, Min A M, and Wa N S 2011 *J. Mater. Sci. Eng. A* **1** 80–86