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SCREENING OF SUSTAINABLE HYDROCARBON EXTRACTED FROM MICROALGAE VIA PHYCOREMEDIATION

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ABSTRACT

A preliminary study of extracted hydrocarbon from microalgae biomass including nutrients removal is presented. The aim of this work is to determine the removal of nutrients from domestic wastewater by *Botryococcus* sp. and to identify the qualitative hydrocarbon from extracted biomass. The results showed that *Botryococcu*s sp. is capable to remove total nitrogen 60.83% and total phosphorus 36.17% from domestic wastewater. Since the best result was found in wastewater treatment, lipid content was performed with solvent extraction using soxhlet extractor. From GC-MS analysis, Phthalic acid, 2-ethylhexyl tridecyl ester was obtained with the largest peak area of 71.56%. This study proved that *Botryococcus* sp. from domestic wastewater treatment phycoremediation could produced biomass with suitable amount of lipids and chemical compound.

Keywords: Microalgae • *Botryococcus* sp. • Domestic wastewater • Nutrients • Hydrocarbon

INTRODUCTION

Domestic wastewater is a main source of contaminations and polluted to waterbodiesas well as aquatic life in marine ecosystem. Current treatment technologies are expensive and partially effective to remove excessive nutrients (McGinn et al. 2012). Therefore, a lot of wastewater researcher had introduced biological treatment using microalgae to deal with the excess nutrients present in wastewater (Abinandan et al. 2013; Ahmed 2014; Al-rajhia et al. 2012; Amini et al. 2013; Arbib et al. 2014; Asmare et al. 2013; Azarpira et al. 2014; Boonchai et al. 2012; Cai et al. 2013; Chan et al. 2013; Choi & Lee 2012; Gani et al. 2015a,b).

Furthermore, growing microalgae in wastewater is not only for treatment purposed, but successfully had been investigated be able to produce bio-oil from microalgae biomass such as hydrocarbon, lipid, biodiesel and biofuel (Yonezawa et al. 2012; Mahapatra & Ramachandra 2013;Abou-Shanab et al. 2013; Cabanelas et al. 2013; Batista et al. 2014; Wu et al. 2014; Mohan et al. 2014; Rajkumar et al. 2014; Gokulan et al. 2013; Rawat et al. 2011; Maity et al. 2014; Woertz et al. 2010; Nascimento et al. 2012; Velichkova 2012; Kothari et al.2013; Órpez et al. 2009; Pittman et al. 2011). Microalgae have high potential as alternative source of biodiesel because it is a sustainable lipid extracted from biological photosynthetic plant. Other than that, microalgae biodiesel also assumed as ecofriendly approach and be able to increase the production of renewable energy to compete with conventional biofuel source (Dragone et al. 2010; Cabanelas et al. 2013; Chekroun & Baghour 2013; Prakash et al. 2014; Surendhiran & Vijay 2012).

Moreover, Bhatt et al. (2014) stated that microalgae gained enormous consideration from scientific community worldwide emerging as a viable feedstock for a renewable energy source virtually being carbon neutral, high lipid content and comparatively more advantageous to other sources of biofuels. Accordingly, wastewaters were chosen to grow microalgae due to high concentration of nutrients and simultaneously effectively to produce biomass for hydrocarbon production. As stated by Wu et al. (2014) where *Botryococcusbraunii* able to produce microalgae lipid content up to 25-80% of dry biomass, *Spirulina maxima* about 6-7% and *Nannochloropsis* sp. up to 68%. Meanwhile for nutrient removal, Sahu 2014 found that total nitrogen could be reduce about 71% and 67% of total phosphorus when he used *Chlorella vulgaris* to treating the municipal wastewater. Other study done by Ji et al. (2013) using *Scenedesmusobliquus* in piggery wastewater found that total nitrogen reduced around 23-58% while for total phosphorus was about 48-69%. In addition, *Botryococcus* sp. reduced total nitrogen 67% in greywater while increased 8.1% in dairy wastewater (Gani et al. 2015a,b). It can be seen that the effective of microalgae in treating wastewater and hydrocarbon mostly depending on the media condition and also selection of microalgae species.

Hence, aim of this paper was to establish the initial steps for the development of new microalgae cultivation system in wastewater to produce sustainable extracted lipid from freshwater microalgae *Botryococcus* sp. biomass. The removal of excessive nutrients such as total nitrogen and total phosphorus present in wastewater were determined. Beside, preliminary hydrocarbon qualitative composition was also performed.

MATERIALS AND METHODS

Microalgae preparation

The *Botryococcus* sp. was collected, isolated and purified prior to the experiment. The microalgal samples were collected from a tropical rainforest South of Peninsular Malaysia. Upon confirmation of the purity of the isolated, the inoculum was kept in modified Bolds basal media (both in agar slant and broth) inside an incubator at ambient temperature (27°C-30°C). The microalgae were subsequently cultured in 2L capacity flask prior to the experiment. After that, the microalgal culture was harvested by centrifugation at low speed (3500rpm) for few minutes with two times washing with sterile distilled water for prior to cell count. The cell density was counted using haemocytometer at an initial cell according to Andersen (2005). After which 10^3cell/mL were inoculated in the wastewater to commence the treatment.

Wastewater sampling

Domestic wastewater was obtained from sewage treatment plant located in BatuPahat, Johor, Malaysia. The samples, which were collected using acid washed 10L bottles (APHA, 2012) and nutrient such as total phosphorus (DR6000, Method 8190) and total nitrogen(TOC Analyzer, Brand: TOC-VCSH, Japan, Shimadzu) were tested immediately. Then, wastewater kept in a cold room (4°C) before commencement the experiment. Prior to the microalgae cultivation process, the wastewater was filtered first with a 0.45µm membrane filter (Whatman).

Cultivation process

The photobioreactor (Figure 1) made of acrylic glass used in this study. The construction of photobioreactor according to Yen et al., (2014) with some modifications. The microalgae cultivated in wastewaters based on the outdoor natural condition. In this concept, wastewater will be circulated and homogenized using a water pump. The total volume of the photobioreactor is approximately 27 L and allowable working volume limited to 25 L only to give air space of photobioreactor. This type of photobioreactor designed to avoid the contamination if compared to the open pond. By that, the purity of the product or hydrocarbon produced can be maintained. Also, some of the advantages using this bioreactor is as more cost-effectively for the growth of microalgae for nutrient removal in wastewater for biomass production, minimal power consumption and easy to handling.

Hydrocarbon Screening Examination

Flocculation method using aluminium sulfate, Al2(SO4)3 as coagulant was employed to harvest the microalgal biomass. Upon concentration of the biomass, oven drying at 60°C for 24 hours followed. After that, extraction procedure was conducted based on EPA Method 9071B (n-Hexane Extractablematerial). This method is applicable for the extraction of non-volatile hydrocarbon, vegetable oils, animal fats, waxes, soaps, grease, biological lipids and related materials.The oil sample was analyzed using DB 5 MS column (30 m x 0.32 mm ID x 0.25 µm film thickness) using GC–MS. The conditions are used as per Dayananda et al.,(2005). The initial temperature of the oven is at 130°C for 5 min thatwas increased to 200°C at the rate of 8°C per minute. After maintaining at 200°C for 2 min, the temperature was increased to 280°C at the rate of 5°C/min and maintained for 15 min. The injector port and the detector temperatures are 240°C and 250°C respectively. The peak was tentatively identified based on library search report or NIST (National Institute of Standard and Technology) database.

RESULT AND DISCUSSION

Nutrients removal

The results shown in Figure 2 clearly indicated a good removal effect of total nitrogen and total phosphorus at final stage of cultivation by *Botryococcus* sp. The removal of total nitrogen was 36.33% on the Day 10 of cultivation and increased up to 60.83% on the Day 20 from initial concentration of 19.9 mg/L. It shown that *Botryococcus* sp. was able to remove more than half of initial concentration the total nitrogen. This result inline with findings of Sahu (2014) and Wang et al. (2014) who reported 71% and 58% removal in total nitrogen using *Chlorella vulgaris* and microalgal bacterial flocs. Meanwhile, total phosphorus removal by *Botryococcus sp.* (Figure 2) slightly lower than total nitrogen where total phosphorus able to be reduce about 16% on the Day 10 and 36.17% on the day 20. However, Sharma & Khan (2013) were observed different removal percentage of total phosphorus in which their *Chlorella sp*. could remove up to 70% on primary treated wastewater. Also, Ji et al. (2013) found that Scenedesmusobliquus capable to remove total phosphorus about 48-69% from piggery wastewater.

There are several factors that had been discussed could give effect to the differential value of removal for both nutrients such as microalgae species and cell concentration used, type of wastewater in term of pollutant load and sampling time, environmental factors like light, photoperiod, temperature, salinity and pH and pretreatment of wastewater before commence the experiment (Komolafe, et al. 2014; Cai et al. 2013; Udom et al. 2013; Zhu et al. 2013; Sacristán de Alva et al. 2013; Kothari et al. 2013; Mata et al. 2012; Sp et al. 2012; Kirrolia et al. 2012; Qin & Li 2006; Creswell 2010; Ugwu et al. 2008). Furthermore, (Bhatt et al. 2014) had stated that microalgae remove significant amount of nitrogen and phosphorus for protein, nucleic acid and phospholipid synthesis of their cell accumulation.

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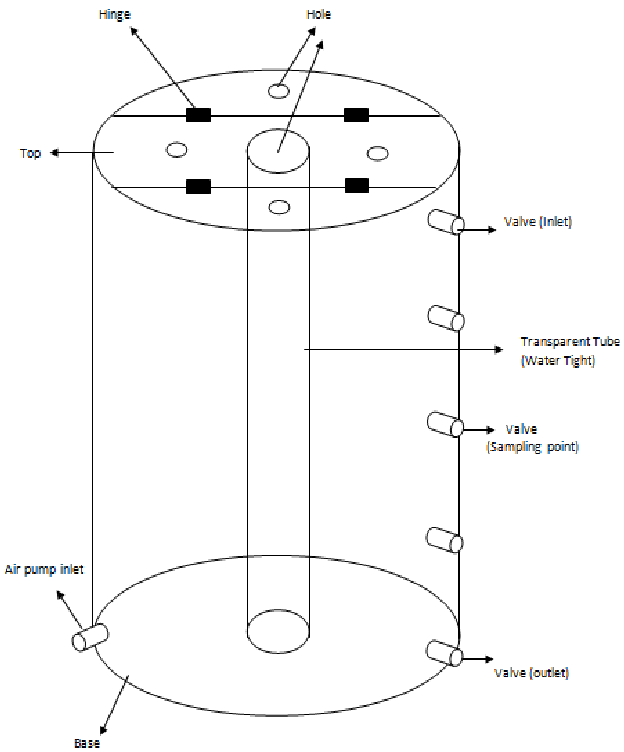

Figure 1 Schematic diagram of photobioreactor system

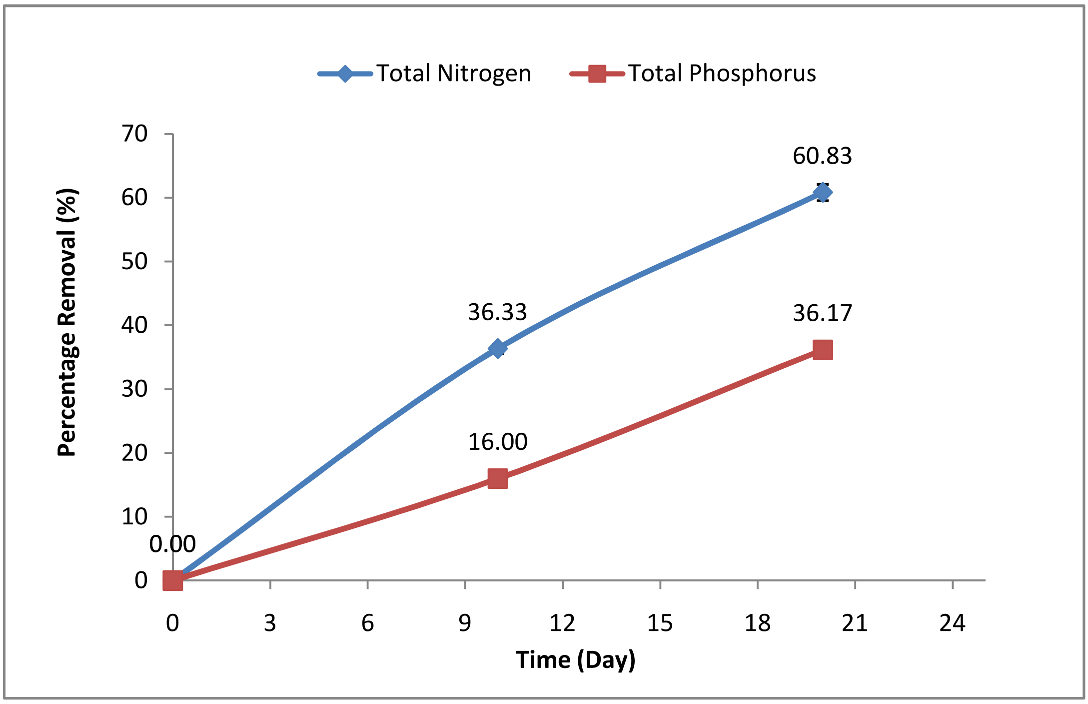

Figure 2 Nutrients removal by *Botryococcus*sp.phycoremediation

Hydrocarbon screening

The qualitative analysis of extracted hydrocarbon from Botryococcus sp. grown on domestic wastewater is tabulated in Table 1 below. Initially, there were 5 compounds were identified in n-Hexane extract by GC-MS. The largest peak area was 71.56% namely Phthalic acid, 2-ethylhexyl tridecyl ester carrying 460.68 g/mol o molecular weight. The second largest peak area 7.59% is due to the presence of 1-Diphenyl (tert-butyl) silyloxy-4-methoxybenzene. The third peak was at 6.85% which is properties of 2-(Acetoxymethyl)-3-(methoxycarbon) biphenylene. The other two less prominent peaks at different area and molecular weight are given in Table 1. This result showed us that the potential of *Botryococcus*sp hydrocarbon in many industry such as biofuel, bio-plastic and bio-based product.Evidence supporting that this oil can be used as abioplastic with the existence of the largest peak area of Phthalic acid. According to Heise and Litz (2004)report which is clearly have indicated the high potential of this oil to be used in plastic industry as additive materials.

Table 1 Hydrocarbon composition from extracted*Botryococcus* sp. biomass

Compounds Name	Molecular Formula	Molecular weight (g/mol)	% Area
Phthalic acid, 2-ethylhexyl tridecyl ester	$C_{29}H_{48}O_4$	460.68	71.56
1-Diphenyl (tert-butyl) silyloxy-4-methoxybenzene	$C_{23}H_{26}O_2Si$	362.53	7.59
2-(Acetoxymethyl)-3-(methoxycarbon) biphenylene	$C_{12}H_8$	152.19	6.85
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15 –hexamethyl-	$C_{16}H_{48}O_7Si_8$	577.23	4.78
Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368.63	3.03

CONCLUSION

Preliminary qualitative screening of hydrocarbon from microalgae is proposed including consecutive steps of phycoremediation, biomass harvesting and lipid extraction by growing *Botryococcus* sp. in photobioreactor. The study proved that *Botryococcus* sp. is effective for the reduction of nutrients from domestic wastewater. Considering that *Botryococcus* sp. have been successfully produced an amount of lipid via phycoremediation in this study, it is enough to suggest that this microalgae is a suitable candidate for combination of hydrocarbon production and wastewater treatment for sustainable development of our environmentalprotection.

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References

Abinandan, S. Premkumar, M. Praveen, K & Shanthakumar, S. (2013). Nutrient Removal From Sewage – An Experimental Study At Laboratory Scale Using Microalgae. International Journal of ChemTech Research, 5(5), pp.2090–2095.

Abitha, R., Sharada, R. & Durgappa, S., (2010). Hydrocarbon Production From Spent Wash And Dairy Waste, Carbondioxide Mitigation and ph Equalization By The Green Alga Botryococcus Braunii. Lake 2010: Wetlands, Biodiversity and Climate Change, (December, 22-24), pp.1–13.

Abou-Shanab, R.A.I., Ji, M., Kim, H., Paeng K.& Jeon, B. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. *Journal of environmental management*, 115, pp.257–64.

Ahmed, S.G.K.A.L., 2014. Dairy Wastewater Treatment Using Microalgae In Karbala City – Iraq. International Journal of Environment, Ecology, Family and Urban Studies (IJEEFUS), 4(2), pp.13–22.

AL-Rajhia, S. Raut, N. AL-Qasmi, F. Qasmi, M and Al Saadi, A. (2012). Treatments of Industrials Wastewater by Using Microalgae.International Conference on Environmental, Biomedical and Biotechnology in Singapore, vol.41 (2012).

Aminia, M. Younesi, H, Lorestani, A.A.Z.&Najafpour, G. (2013). Determination of optimum conditions for dairy wastewater treatment in UAASB reactor for removal of nutrients. *Bioresource technology*, 145, pp.71–9.

Andersen, R.A., 2005. *Algal Culturing Techniques* 1st Editio., Burlington, USA: ELSEVIER Academic Press.

Arbib, Z. et al. (2014). Capability of different microalgae species for phytoremediation processes: wastewater tertiary treatment, CO2 bio-fixation and low cost biofuels production. *Water research*, 49, pp.465–74.

Asmare, A.M., Demessie, B.A. & Murthy, G.S., 2013. Baseline Study on the Dairy Wastewater Treatment Performance and Microalgae Biomass Productivity of an Open Pond Pilot Plant : Ethiopian Case. Journal of Algal Biomass Utilisation, 4(4), pp.88–109.

Azarpiral, H., Behdarvand, P., Dhumal, K. & Pondhe1, G. (2014). Potential use of cyanobacteria species in phycoremediation of municipal wastewater. , 4(4), pp.105–111.

Batista, A.P. et al. (2014). Combining urban wastewater treatment with biohydrogen production – an integrated microalgae-based approach. *Bioresource Technology*. Article in press.

Bhatt, N.C. Panwar, A. Bisht, T.S. & Tamta, S. (2014). Coupling of algal biofuel production with wastewater. *Scientific World Journal*, 2014, p.210504.

Boonchai Rawiwan, Seo Gyu Tae, Park Da Rang & Seong, Chung Yeol (2012). Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2(6), pp.407–410.

Cabanelas, I.T.D. et al. (2013). From waste to energy: Microalgae production in wastewater and glycerol. *Applied Energy*, 109, pp.283–290.

Cai, T., Park, S.Y. & Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, 19, pp.360–369.

Chan, A., Salsali, H. & Mcbean, E. (2013). Heavy Metal Removal (Copper and Zinc) in Secondary E ffl uent from Wastewater Treatment Plants by Microalgae.ACS Sustainable Chemistry & Engineering. Doi: dx.doi.org/10.1021/sc400289z.

Chekroun, K. Ben & Baghour, M. (2013). The role of algae in phytoremediation of heavy metals : A review. J. Mater. Environ. Sci. 4(6), pp.873–880.

Choi, H. & Lee, S., 2012. Effects of Microalgae on the Removal of Nutrients from Wastewater : Various Concentrations of Chlorella vulgaris. Environmental Engineering Research, 17, pp.3–8.

Creswell, L., 2010. Phytoplankton Culture for Aquaculture Feed. SRAC Publication No. 5004 September 2010.

Dayananda, C., Sarada, R., Bhattacharya, S. & Raviishankar, G.A. (2005). Effect of media and culture conditions on growth and hydrocarbon production by Botryococcus braunii. *Process Biochemistry*, 40(9), pp.3125–3131.

Dragone, G., Fernandes, B., Vucente, A.A. & Teixeira, J.A. (2010). Third generation biofuels from microalgae. *Applied Microbiology and Microbial biotechnology*. Mendez-Vilas A (ed), Formate*x*, pp.1355–1366.

Gani, P., Sunar, N.M., Latiff, A.A., Kamaludin, N.S., et al. (2015a). Experimental Study for Phycoremediation of Botryococcus sp . On Greywater. *Applied Mechanics and Materials*, 773-774(2015), pp.1312–1317.

Gani, P., Sunar, N.M., Latiff, A.A., Joo, I.T.K., et al. (2015b). Phycoremediation of Dairy Wastewater by Using Green Microalgae : Botryococcus sp . *Applied Mechanics and Materials*, 773-774(2015), pp.1318–1323.

Gokulan, R., Sathish, N. & Kumar, R.P. (2013). Treatment Of Grey Water Using Hydrocarbon Producing Botryococcus braunii. *International Journal of ChemTech Research*, 5(3), pp.1390–1392.

Heise, S. and Litz, N. (2004). Phthalates. German Federal Environmental Agency, Berlin, Germany.

Ji, M., Reda, A.I., Abou-Shanab, Hwang, J., Timmes, T.C., Kim, H., Oh, Y. & Jeon, B. (2013). Removal of Nitrogen and Phosphorus from Piggery Wastewater Effluent Using the Green Microalga Scenedesmus obliquus. Journal of Environmental Engineering, (139) pp.1198–1205.

Kothari, R., Prasad, R., Kumar, V. & Singh, D. P. (2013). Production of biodiesel from microalgae Chlamydomonas polypyrenoideum grown on dairy industry wastewater. *Bioresource technology*, 144, pp.499–503

Kotowska, U., Biegańska, K. & Isidorov, V.A. (2012). Screening of Trace Organic Compounds in Municipal Wastewater by Gas Chromatography-Mass Spectrometry. , 21(1), pp.129–138.

Mahapatra, D.M. & Ramachandra, T. V. (2013). Algal biofuel : bountiful lipid from Chlorococcum sp . proliferating in municipal wastewater. Current Science, Vol. 105, No. 1, pp.47–56.

Maity, J.P. et al., 2014. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives - A mini review. *Energy*. http://dx.doi.org/10.1016/j.energy.2014.04.003 (In Press).

McGinn, P.J. et al., 2012. Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode. *Algal Research*, 1(2), pp.155–165.

Mohan, S.V. et al. (2014). Algae Oils as Fuels. In *Biofuel from Algae*. USA: Elsevier B.V., pp. 155–187.

Nascimento, I.A. et al (2012). Screening Microalgae Strains for Biodiesel Production: Lipid Productivity and Estimation of Fuel Quality Based on Fatty Acids Profiles as Selective Criteria. *BioEnergy Research*, 6(1), pp.1–13.

Órpez, R. et al. (2009). Growth of the microalga Botryococcus braunii in secondarily treated sewage. *Desalination*, 246(1-3), pp.625–630.

Pittman, J.K., Dean, A.P. & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource technology*, 102(1), pp.17–25.

Prakash, J. et al. (2014). Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review.

Rajkumar, R., Yaakob, Z. & Takriff, M.S. (2014). Potential of the Micro and Macro Algae for Biofuel. *BioResources*, 9(1), pp.1–29.

Rawat, I., Kumar, R.R., Mutanda, T. & Bux, F. (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy*, 88(10), pp.3411–3424

Sahu, O. (2014). Reduction of Organic and Inorganic Pollutant from Waste Water by Algae. *International Letters of Natural Sciences*, 8(1), pp.1–8.

Sharma, G.K. & Khan, S.A. (2013). Bioremediation of Sewage Wastewater Using Selective Algae for Manure Production. *International Journal of Environmental Engineering and Management*, 4(6), pp.573–580.

Surendhiran, D. & Vijay, M. (2012). Microalgal Biodiesel - A Comprehensive Review on the Potential and Alternative Biofuel. , 2(11), pp.71–82.

Ugwu, C.U., Aoyagi, H. & Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology*, 99(10), pp.4021–4028.

Velichkova, K.. (2012). Cultivation of Botryococcus braunii strain in relation of its use for biodiesel production. , J. BioSci. Biotech.(Special Edition) pp.157–162.

Wang, H.-Y., Bluck, D. & Van Wie, B.J. (2014). Conversion of microalgae to jet fuel: process design and simulation. *Bioresource technology*, 167, pp.349–57.

Woertz, I., Feffer, A., Lundquist, T. & Nelson, Y. (2010). Algae Grown on Dairy and Municipal Wastewater for Biofuel Feedstock.*Journal Of Environmental Engineering* , 135(11), pp. 1115-1122.

Wu, Y.H. et al. (2014). Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review. *Renewable and Sustainable Energy Reviews*, 33, pp.675–688.

Yen, H. et al. (2014). Design of Photobioreactors for Algal Cultivation. In *Biofuel from Algae*. Elsevierr Inc., pp. 23–45.

Yonezawa, N. et al. (2012). Effects of soybean curd wastewater on the growth and hydrocarbon production of Botryococcus braunii strain BOT-22. *Bioresource technology*, 109, pp.304–7.