EFFECT OF PHOTOPERIOD ON THE GROWTH OF *CHLAMYDOMONAS INCERTA* AND POLLUTANT REMOVAL

Mazen Abdo Alqadi, Shazwin Mat Taib*, Mohd Fadhil Md Din & Hesam Kamyab

Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, UTM Skudai 81310, Johor, Malaysia

*Corresponding Author: *Shazwin@utm.my*

Abstract: The key to an economic and healthy algal culture is to optimize the growth conditions. The main objective of this research was to investigate the effects of photoperiod (12:12, 16:8 and 24:0 light: dark cycle) on the growth of indigenous microalgae *Chlamydomonas incerta (C. incerta)* when cultured in Palm Oil Mill Effluent (POME). POME is nutrient-rich wastewater and one of the highest organic content that could enhance formation of algal bloom. The amount of light intensity and photoperiod has major influence on biomass productivity and photosynthesis process. Microalgae cultures were grown at room temperature and subjected to light source with intensity of ±100 mol m^{-2} s^{-1}. The growth rate was evaluated based on Optical Density (OD) measured every alternate day during 17 days. Whereas pollutant removal's ability was determined based on reduction in Chemical Oxygen Demand (COD) of POME. This study revealed that, *C. incerta*is a mixotrophic microalga because 12:12 light: dark cycle has resulted in the highest biomass concentration (0.786 g L^{-1}) which is eight times the initial concentration and similarly biomass productivity and specific growth rate, 0.04 g $L^{-1}d^{-1}$ and 0.118 d^{-1}, respectively. The highest pollutants removal was achieved at 12:12 L:D cycle with 70 % COD removal. These result specified that the photoperiod condition has notable impacts on adjusting pollutant removal and producing microalgal biomass. Therefore, the control of photoperiod was proposed as a significant operating parameter in the microalgal wastewater treatment.

Keywords: *Photoperiod, Chlamydomonas incerta, microalgae, POME*

1.0 Introduction

Microalgae have been a major focus of attention for biomass production since 1950 (Grobbelaar, 2000). Microalgae are minute photosynthetic organisms that thrive in diverse habitats, mostly aquatic environments, and which are capable of converting light energy and a source of carbon, carbon dioxide (CO_2), into a set of organic material or "biomass" (Wen and Johnson, 2009). They are unicellular species, prokaryotic or eukaryotic, photosynthetic micro-organisms which can grow rapidly in suspension,

mainly in aqueous solutions and live in difficult conditions, commonly found in marine and freshwater with a size ranging from a few micrometers to a few hundreds of micrometers (Selmani *et al*., 2013; Venkatesan *et al*., 2015). Microalgae utilize light energy in the photosynthesis process which converts inorganic material into sampler sugar as a source of energy for cell metabolism. Microalgae are more efficient than plants in the process of photosynthesis (Khan *et al*., 2009).

Environmental parameters such as light source and intensity, photoperiod, temperature, salinity, pH and mixing influence the growth of microalgae (Atta *et al*., 2013; Singh *et al*., 2015a). Thus, it is recommended to optimize and maintain these parameters during the cultivation period. The growth and the circadian rhythm of photosynthesis in algal cells depend on the length of photoperiod and vary from species to species. Light: dark (L/D) cycles have great influence on nutrient metabolism and synthesis of organic compounds. Therefore, microalgae growth rate and biomass productivity could be increased and decreased depending on the period of exposure to light (Singh *et al*., 2015b). Changing the L: D cycle timescales may involve different interactions with cell metabolism for instance: interaction with the primary mechanism of photon absorption in the photosynthetic chain, limitation of photo inhibition effects, by reducing exposure to high irradiance periods to then reduce degradation of the photosynthetic apparatus, and interaction with the overall energy metabolism of the microalgae (Takache *et al*., 2015). Produced microalgae cells are directly proportional to the length of exposure to light, and hence relatively bigger cells sizes under L/D cycle could be obtained. Besides, L/D cycle system offers higher efficiency in terms of organic carbon, phosphorus and nitrate removal (Lee and Lee, 2001).

Culturing microalgae in wastewater provide a low-cost tertiary treatment method and simultaneously enhance the production of microalgae biomass by providing nutrients and organic compound necessary for microalgae metabolism. Organic load of effluent decreases due to metabolism and uptake of the wastewater components by microalgae. This is considered a win-win strategy by Lam and Lee, (2011) in term of economic and environmental sustainability. In Malaysia, POME occurred to be best type of wastewater for this strategy. It has high concentration of carbohydrate, proteins, nutrient (nitrogen and phosphorus), lipid and minerals (Saenge, 2010; Wu *et al.,* 2009) which make it the most suitable raw material for bioconversion by biotechnological means. Moreover, palm oil mill represents an important economical provider to Malaysian government where it provides 9.8 percent of the total revenue, source of livelihood to rural families and employment opportunities to agricultural workers (Ching *et al*., 2015; Lam and Lee, 2011). From microalgae that naturally live in POME, *Chlamydomonas* has been found to grow faster and perform better in pollutant removal (Kamyab *et al*., 2015).

The aim of this research is to investigate the effects of three different photoperiods on the biomass concentration of *C. incerta* and removal of pollutants from POME based on Chemical Oxygen Demand (COD). Overall, the results of this study are important to

introduce optimal light conditions correspond to the highest microalgae growth and pollutant removal.

2.0 Materials and Methods

2.1 Microalgae Strain and Culture Medium

C. incerta with catalogue number KR349061 (Kamyab *et al*., 2016b) was obtained from previous research work from the Environmental Laboratory, Faculty of Civil Engineering, Universiti Teknologi Malaysia. A sample of 100 mL with optical density of 0.5 nm was initially cultured in Bold's Basel Medium (BBM) (Stein, 1975) containing (g L^{-1}) KH_2PO_4, 17.5; $CaCl_2.2H_2O$, 2.5; $MgSO_4.7H_2O$, 7.5; $NaNO_3$, 25; K_2HPO_4, 7.5; NaCl, 2.5; Na_2EDTA, 10; KOH, 6.2; $FeSO_4.7H_2O$, 4.98; H_2SO_4 (Conc.) [1 $mL.L^{-1}$]; H_3BO_3, 2.86. Furthermore, pH value, initially was in a range of 6.4 to 7.2, was controlled using HCL and NaOH solutions. After that, the BBM solution was autoclaved at 121°C for 15 min in order to kill infectious agents and ensure no contamination in the solution.

2.2 POME

The raw POME sample was collected from facultative ponds at Felda Palm Oil Industries Sdn Bhd (1° 44' 0.00" N:103° 39' 0.00" E) in Kulai Johor Bahru. The obtained sample was stored in plastic containers with proper labels and well preserved in a cool room at 4 ℃ to prevent any contamination and to limit the activity of biodegradation process (Kamyab *et al*., 2014b, Kamyab *et al*., 2014a). Large and bulky materials in raw POME sample were removed before dilution (Kamyab *et al.*, 2014b). For analysis convenience, serial dilution was carried out using distilled water. Prior to sample preparation for microalgae cultivation, POME sample was left over for two hours in order to return the POME to room temperature and analyzed for Chemical Oxygen Demand (COD) (Kamyab *et al.*, 2014b).

2.3 Experimental Setup and Condition

The experiment was conducted in an environmental chamber equipped with white fluorescent lamps (Phillips) of 100 µmol $m^{-2}s^{-1}$ intensity at a room temperature (24 – 28 ℃). Prior to the injection of *C. incerta* (10% v:v), the POME used as a medium was diluted to a final concentration of 250 mg L^{-1} COD and adjusted to a final pH of 7.15. Three lamps were connected to an electronic automatic switch equipped with timer to apply 24:0, 16:8 and 12:12 hours light: dark cycle. After setting up the cultivation condition, the stock culture (with optical density of 0.35 nm) was inoculated into each 500 mL Erlenmeyer culture flask to get 10% (v/v) inoculum density. Three replications

were used for the all cultures and control media. The cell concentration was monitored every alternative day by measuring OD at 600 nm.

2.4 Analytical Method

2.4.1 POME Analysis

The characteristics of the raw POME such as COD were determined immediately after sample collection and after treatment with *C. incerta* at the end of the cultivation period according to the Standard Methods for the Examination of Water and Wastewater (APHA) (Eaton *et al*., 2005).

2.4.2 Microalgae Growth

The effects of photoperiod on the growth of *C. incerta* were monitored by measuring OD at every alternate day. Optical Density (OD) was measured using HACH DR6000 spectrophotometer at a single wavelength of 600 nm (Feng *et al*., 2011; Hadiyanto and Nur, 2012; Kamyab *et al*., 2016b; Nurul-Adela *et al*., 2016; Putri *et al*., 2011; Sukumaran *et al*., 2014). The result was recorded and tabulated for further analysis. The sample is returned back to the flask once the measurement is done.

2.4.3 Kinetic Parameters

The specific growth rate (μ) was determined by the following equation (Putri *et al*., 2011, Ponraj and Din, 2013, Wahidin *et al*., 2013):

$$Specific\ Growth\ Rate\ (\mu) = \frac{1}{t}\ln(\frac{X_2}{X_1}) \qquad \text{Equation 1}$$

Where it is the time of cultivation run (days) and X_2 and X_1 are the biomass concentration at the end and beginning of cultivation, respectively. Whereas the biomass productivity was calculated by the equation (Ponraj and Din, 2013, Sukumaran *et al*., 2014, Jacob-Lopes *et al*., 2009):

$$\text{Biomass Productivity (P)} = \frac{X_2 - X_1}{t} \qquad \text{Equation 2}$$

3.0 Results and Discussion

3.1 Effect of Photoperiod on the Growth of C. incerta

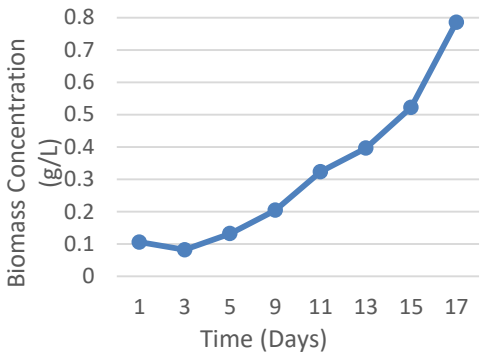
The growth rate and biomass productivity of *C. incerta* were investigated under three different light: dark cycles. Figure 1, shows the effect of photoperiod on the growth of *C.*

incerta. According to Figure 1, all three cycle spend three days for the adaptation phase. In the third day, the growth curve experiences a slight increase which represent the start of the exponential phase. Moreover, from the graph, it is clear that this strain has the capacity to grow further but at varying percentage with respect to the L:D cycle.

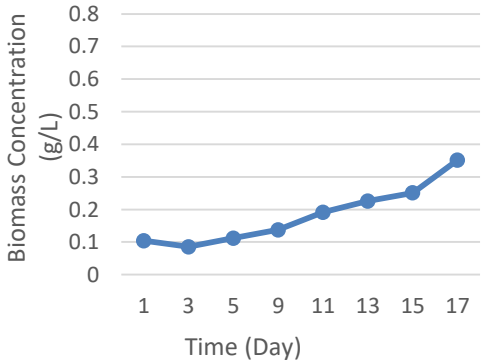
Even though, all light: dark cycles showed a positive growth, 12:12 L:D cycle showed a better growth compared to 16:8 and 24:0. The maximum biomass obtained at the 17^{th} day of cultivation was 0.8 (g L^{-1}) at 12:12 L:D cycle whereas 16:8 and 24:0 L:D cycle achieved a growth of 0.35 g L^{-1} (Figure 1). In contrast, *Chlorella pyrenoidosa (CP)* reached a maximum growth at 24:0 L:D cycle (0.046 g L^{1}) followed by0.027 and 0.03 g L^{-1} for 16:8 and 8:16 L:D cycle, respectively (Kamyab*et al*., 2016a). However, that does not mean CP perform better in POME medium as it reached these maximum values prior to the death phase whereas *C.inserta* reaches these values at exponential phase on 17^{th} day of ongoing experiment.

The different cell growth profiles for *C. incerta*, with different light cycles as might be seen as a function of the duration of their exposure to light. The cultures grown under a photoperiod of 16:8 was found similar to the growth under 24:0 light: dark cycle. Moreover, Wahidin*et al*.(2013), obtained similar trend of photoperiod effect with the microalgae strain *Nannochloropsis sp*., reporting that the high cell concentration can only reached 4.8 * 10^7 cell mL^{-1} under a photoperiod of 12:12 light: dark cycles on the 8-day cultivation. However, under 24: 0 h, the cell concentration the result was 3.6 *10^7 cell mL^{-1}. This could be due to the fact that growth at higher light intensity was faster and as a result to that, it does not require extended photoperiod cycle where higher light intensities can lead to photo inhibition. Some microalgae species could show preferences with respect to the duration of the light periods, resulting from the environmental conditions in which they were isolated in nature (Jacob-Lopes *et al*., 2009). In addition, the effect of the photoperiod has been reported as a key element in photosynthetic activity and in the growth rates of microalgae (Janssen *et al*., 2000, Janssen *et al*., 2001).

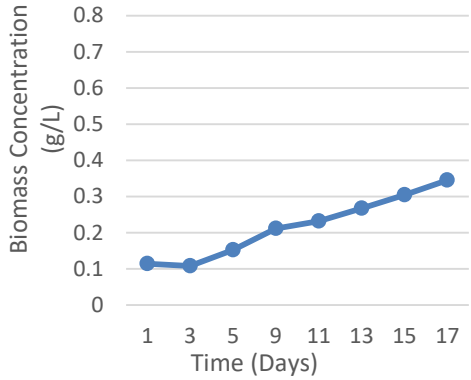
The biomass productivity and specific growth rate showed similar result to the biomass concentration. The maximum biomass productivity and specific growth rate were achieved at the 12:12 L: D cycle with the value of 0.04 g $L^{-1}d^{-1}$, 0.118 respectively. Whereas for 16/8 and 24/0 L/D cycle, the biomass productivity and specific growth rate were (0.015 g $L^{-1}d^{-1}$ and 0.072) and (0.014 g $L^{-1}d^{-1}$ and 0.065), respectively (Table 1). Similar trend were reported by (Jacob-Lopes *et al*., 2009) in which 12:12 L:D cycle achieved the highest biomass productivity and cell density. These results are related to the fact that the cultures were maintained and propagated under a 12 h light cycle, resulting in an improvement in the volumetric growth rate and maximum cell concentrations under these conditions. Moreover, Light above a saturation point causes light inhibition.



12:12



16:8



24:0

Figure 1: Effect of photoperiod on biomass concenration

Table 1: Effect of photoperiod on the biomass and pollutant removal.

Photoperiod	12:12	16:8	24:0
Biomass productivity (P)	0.04	0.015	0.014
Specific Growth Rate (μ)	0.118	0.072	0.065
COD Removal (%)	70	60	52

This study showed that proper lighting system is crucial for microalgae to sustain cell growth (McGinnis *et al*., 1997, Lehr and Posten, 2009) and in this case *C. incerta*. The optical density of microalgae grown in POME increased steadily (Figure 1) as expected as POME is rich with nutrient. On the eleventh day of cultivation, the biomass attached to the conical flask was removed manually so that it is fully homogenized which resulted in a better increase in the OD afterward. Thus, good mixing is necessary to prevent the algal cells from settling down. With adequate mixing, nutrients can be evenly distributed in the medium thereby disrupting diffusion barriers at the algal cell surfaces. Moreover, sufficient mixing of the medium can uniformly expose the algal cells to the light source, ensure quick removal of the oxygen produced by the microalgae during photosynthesis and subsequently avoid potential oxidative stress on the algal cells (Guo *et al*., 2015).

3.2 Effect of Photoperiod on the Removal of Pollutant by C.incerta

Microalgae in general have been reported by many researchers to have high potential in wastewater treatment. The main goal of this research was to assess the efficiency of *C. incerta* in reducing COD. The initial COD in the diluted POME sample prior to the cultivation was 250 mg L^{-1}. Based on Table 1, *C. incerta* was found to be more effective in reducing the pollutants concentration in POME when subjected to a photoperiod 12:12 L: D cycle with removal efficiency of 70 %. These results are in agreement with the result obtained by Kamyab *et al*.(2015) where it stated that, for a POME medium with initial COD concentration of 250 mg L^{-1} COD, the highest removal efficiency of COD by *C. incerta* was 67.35 %. Moreover, Travieso *et al*.(2006),reported that, *chlorella vulgaris* can reduce the COD concentration by 88% when cultured in piggery wastewater with initial COD value of 250 mg/L. In addition, Kamyab *et al*.(2014)reported that cultivation of mixed micro-macro algae in a 250 mg/L COD POME can result in the 71.16 % reduction in COD. Therefore, this study revealed that *C. incerta* could be a mixotrophic species and may grow better at 12:12 L: D cycle.

4.0 Conclusions

L/D cycles are often discussed as a way to improve photosynthetic conversion and the resulting biomass productivity. Experiments conducted here made it possible to study

the L/D cycle effect on photosynthetic growth of microalga *C. incerta* as well as on the removal of pollutant from POME. The results revealed the growth of *C. incerta* could be increased when the culture is subjected to a 12:12 L: D cycle at light intensity of 100 μmol s^{-1} m^2) at room temperature. In contrast, *C. incerta* showed a lower cell density and specific growth rate when the photoperiod cycle was extended to 16:8 and 24:0 h light exposure at the same light intensity. Results indicated improved specific growth rates and biomass concentration are accompanied by improved pollutant removal where the highest percentage of COD removal was obtained at the maximum biomass concentration and specific growth rate which was obtained at 12:12 L: D cycle. The highest exposure period to light have less growth of *C. incerta* and pollutant removal. *C. incerta* has shown to be a mixotrophic strain when cultured in POME of 250 mg L^{-1} COD concentration. Overall, these results indicated that the photoperiod condition has notable impacts on adjusting nutrient removal and producing microbial biomass. Therefore, the control of photoperiod was suggested as an important operating parameter in the algal wastewater treatment. For future research, *C. incerta* may be considered as an appropriate microalgae species for treating other sources of wastewater because microalgae can be easily grown in POME and is abundantly present throughout the year in Malaysia. Furthermore, it can be considered to produce biodiesel due to its fast growth and high lipid content.

5.0 Acknowledgements

The authors acknowledge the research grants provided by Look East Policy (R.J1 3000. 7822. 44148), UTM Research University Grant (Q. J1 3000. 2517. 10H25) and FRGS (R. J13000.2822. 4F474).The authors are also grateful for the generous support from Ministry of Higher Education -Yemen.

References

Atta, M., Idris, A., Bukhari, A. &Wahidin, S. (2013). Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. *Bioresource Technology,* 148**,** 373-378.

Ching, J. W., Cheahb, Tau, L., Pau, S., Joon, J. &Jo-Shu, C. (2015). Algae Cultivation in Wastewater for Biodiesel A Review *Chemical Engineering Transactions,* 45. pp. 1393-1398.

Eaton, A., Clesceri, L. S., Rice, E. W., Greenberg, A. E. &Franson, M. (2005). APHA: standard methods for the examination of water and wastewater. *Centennial Edition., APHA, AWWA, WEF, Washington, DC*.

Feng, Y., LI, C. &Zhang, D. (2011). Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. *Bioresource Technology,* 102**,** 101-105.

Grobbelaar, J. U. 2000. Physiological and technological considerations for optimising mass algal cultures. *Journal of Applied Phycology,* 12**,** 201-206.

Guo, Z., Phooi, W. B. A., Lim, Z. J. &Tong, Y. W. (2015). Control of CO_2 input conditions during outdoor culture of Chlorella vulgaris in bubble column photobioreactors. *Bioresource Technology,* 186**,** 238-245.

Hadiyanto &Nur, M. M. A. (2012). Potential of Palm Oil Mill Effluent (POME) as Medium Growth of Chlorella sp for Bioenergy Production. *Potential of Palm Oil Mill Effluent (POME) as Medium Growth of Chlorella sp for Bioenergy Production,* 3**,** 67-74.

Jacob-Lopes, E., Scoparo, C. H. G., Lacerda, L. M. C. F. &Franco, T. T. (2009). Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing: Process Intensification,* 48**,** 306-310.

Janssen, M., Janssen, M., DeWinter, M., Tramper, J., Mur, L. R., Snel, J. &Wijffels, R. H. (2000). Efficiency of light utilization of Chlamydomonas reinhardtii under medium-duration light/dark cycles. *Journal of Biotechnology,* 78**,** 123-137.

Janssen, M., Slenders, P., Tramper, J., Mur, L. R. &Wijffels, R. (2001). Photosynthetic efficiency of Dunaliella tertiolecta under short light/dark cycles. *Enzyme and Microbial Technology,* 29**,** 298-305.

Kamyab, H., Din, M. F. M., Ghoshal, S. K., Lee, C. T., Keyvanfar, A., Bavafa, A. A., Rezania, S. &Lim, J. S. (2016a). Chlorella pyrenoidosa mediated lipid production using Malaysian agricultural wastewater: effects of photon and carbon. *Waste and Biomass Valorization***,** 1-10.

Kamyab, H., Din, M. F. M., Keyvanfar, A., Majid, M. Z. A., Talaiekhozani, A., Shafaghat, A., Lee, C. T., Shiun, L. J. &Ismail, H. H. (2015). Efficiency of Microalgae Chlamydomonas on the Removal of Pollutants from Palm Oil Mill Effluent (POME). *Energy Procedia.*

Kamyab, H., Din, M. F. M., Tin, C. L., Ponraj, M., Soltani, M., Mohamad, S. E. &Roudi, A. M. (2014a). Micro-Macro Algal Mixture as a Promising Agent for Treating POME Discharge and its Potential Use as Animal Feed Stock Enhancer. *Jurnal Teknologi,* 68.

Kamyab, H., Md Din, M. F., Lee, C. T., Keyvanfar, A., Shafaghat, A., Majid, M. Z. A., Ponraj, M. &Yun, T. X. (2014b). Lipid production by microalgae Chlorella pyrenoidosa cultivated in palm oil mill effluent (POME) using hybrid photo bioreactor (HPBR). *Desalination and Water Treatment***,** 1-13.

Kamyab, H., Md Din, M. F., Ponraj, M., Keyvanfar, A., Rezania, S., Taib, S. M. &Abd Majid, M. Z. (2016b). Isolation and screening of microalgae from agro-industrial wastewater (POME) for biomass and biodiesel sources. *Desalination and Water Treatment***,** 1-8.

Khan, S. A., Hussain, M. Z., Prasad, S. &Banerjee, U. (2009). Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews,* 13**,** 2361-2372.

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

Lee, K. &Lee, C.-G. (2001). Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering,* 6**,** 194-199.

Lehr, F. &Posten, C. (2009). Closed photo-bioreactors as tools for biofuel production. *Current Opinion in Biotechnology,* 20**,** 280-285.

Mcginnis, K. M., Dempster, T. A. &Sommerfeld, M. R. (1997). Characterization of the growth and lipid content of the diatom Chaetoceros muelleri. *Journal of Applied Phycology,* 9**,** 19-24.

Nurul-Adela, B., Nasrin, A.-B. &Loh, S.-K. (2016). Palm oil mill effluent as a low-cost substrate for bioflocculant production by Bacillus marisflavi NA8. *Bioresources and Bioprocessing,* 3**,** 1.

Ponraj, M. &Din, M. F. M. (2013). Effect of light/dark cycle on biomass and lipid productivity by Chlorella pyrenoidosa using palm oil mill effluent (POME). *J. Sci. Ind. Res,* 72**,** 703-706.

Putri, E. F., M., Ahmed, S., Jamaluddin, H. &Chelliapan, S. (2011). Investigation of microalgae for high lipid content using palm oil mill effluent (POME) as carbon source. *2011 International Conference on Environment and Industrial Innovation.* Singapore: IACSIT Press.

Saenge, C. (2010). *Potential use of Oleaginous Rhodotorula glutinis for bioconversion of palm oil mill effluent and crude glycerol to oil feedstock for biodiesel production.* Doctor of Philosophy, Songkla University.

Selmani, N., Mirghani, M. E. S. &Alam, M. Z. (2013). Study the Growth of Microalgae in Palm Oil Mill Effluent Waste Water. *4th International Conference on Energy and Environment 2013 (Icee 2013),* 16.

Singh, P., Gupta, S. K., Guldhe, A., Rawat, I. &Bux, F. (2015a). Chapter 4 - Microalgae Isolation and Basic Culturing Techniques. *In:* KIM, S.-K. (ed.) *Handbook of Marine Microalgae.* Boston: Academic Press.

Singh, P., Gupta, S. K., Guldhe, A., Rawat, I. &Bux, F. (2015b). Chapter 4 - Microalgae Isolation and Basic Culturing Techniques A2 - Kim, Se-Kwon. *Handbook of Marine Microalgae.* Boston: Academic Press.

Stein, J. R. (1975). Handbook of Phycological Methods: Culture Methods and Growth Measurements, Cambridge University.

Sukumaran, P., Nulit, R., Zulkifly, S., Halimoon, N., Omar, H. &Ismail, A. (2014). Potential of fresh POME as a growth medium in mass production of Arthrospira platensis. *Int. J. Curr. Microbiol. App. Sci,* 3**,** 235-250.

Takache, H., Pruvost, J. &Marec, H. (2015). Investigation of light/dark cycles effects on the photosynthetic growth of Chlamydomonas reinhardtii in conditions representative of photobioreactor cultivation. *Algal Research,* 8**,** 192-204.

Travieso, L., Benítez, F., Sánchez, E., Borja, R., Martín, A. &Colmenarejo, M. (2006). Batch mixed culture of Chlorella vulgaris using settled and diluted piggery waste. *Ecological Engineering,* 28**,** 158-165.

Venkatesan, J., Manivasagan, P. &Kim, S.-K. (2015). Chapter 1 - Marine Microalgae Biotechnology: Present Trends and Future Advances. *In:* KIM, S.-K. (ed.) *Handbook of Marine Microalgae.* Boston: Academic Press.

Wahidin, S., Idris, A. &Shaleh, S. R. M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. *Bioresource Technology,* 129**,** 7-11.

Wen, Z. &Johnson, M. B. (2009). Microalgae as a feedstock for biofuel production.

Wu, T. Y., Mohammad, A. W., Jahim, J. M. &Anuar, N. (2009). A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnology Advances,* 27**,** 40-52.