Design of a Pilot Scale Outdoor Photobioreactor for Mass Cultivation of Local Microalga

Rachel Fran Mansa, Azrinah Tahir, Lu Mee Hua, Jedol Dayou, Coswald Stephen Sipaut

***Abstract*—**Presently microalgae is considered as an alternative biodiesel source and have been cultivated in large scale for commercial use. However, there is lack of efficient systems which utilize solar energy effectively for mass cultivation of microalgae. In this study, a scaled up 280 L flat panel airlift photobioreactor (FP-ALPBR), based on previous work by Issarapayup and co-workers [1], was designed and constructed fiberglass as an alternative system for the large scale outdoor cultivation of microalgae in Malaysia. A local strain, Chlorella sp. was used to assess the growth productivity. The 280 L FP-ALPBR was capable of giving cell productivity of 2.63×105 cells ml-1 d-1, maximum cell density of 6.01×106 cells ml-1 and specific growth rate of 0.15 day-1. The performance of this photobioreactor was compared with the 17 L FP-ALPBR and 90 L FP-ALPBR of the same design. The 280 L FP-ALPBR gave a better performance in terms of maximum cell density, but as expected for large scale it resulted in a considerable decrease in specific growth. This photobioreactor was found to produce a larger harvesting volume and cell density but could not compare in growth rate produced by the smaller 17 L FP-ALPBR and the 90 L FP−ALPBR.

***Keywords*—**Microalgae, Chlorella sp., Photobioreactor

I. Introduction

MICROALGAE consist of a large group of photosynthetic microorganisms ranging from the prokaryotic cells to the eukaryotic cells type. Generally, their structure is varied from a unicellular to simple multi-cellular structure. These are the unique characteristics that enable microalgae to grow rapidly and survive in almost everywhere in the ecosystem, includes terrestrial, aquatic, and even in the severe environment. Reference [2] suggested that more than 50,000 species of microalgae exist, but only a limited number of around 30,000 have that have been studied and analyzed [2].

Lately, many research reports and articles have described various advantages of microalgae, especially for the biodiesel production [3]-[5]. The researchers suggested that microalgae have shown promising results compared to other available feedstock. This is because microalgae are robust, and easy to cultivate. It was found that the cultivation of microalgae does not require specific nutrients for growth [3]-[10]. Hence systems utilizing wastewater and flue gas had been proposed to be used to increase the productivity of the microalgae cultivation [1]-[2]. Compared to conventional agricultural crops, and other aquatic plants, microalgae can grow at much higher rates with much higher oil productivity, and even much less land area. The land area required to grow microalgae is up to 49 or 132 times less when compared to rapeseed or soybean crops, for a 30% (w/w) of oil content in algae biomass [3].

Rachel Fran Mansa, Azrinah Tahir, Lu Mee Hua, Jedol Dayou and Coswald Stephen Sipaut are attached with Energy and Minerals Research Group, Minerals and Materials Research Unit, School of Engineering and Information Technology, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia. Corresponding author email: rfmansa@ums.edu.my

A closed system produces much higher cell productivity and enables a better control compared to open system [13; 14]. A photobioreactor is the most commonly used closed system, it consist of a closed (or mostly closed) vessel which uses some type of light source to provide photonic input into the reactor for phototropic production. With closed photobioreactors, higher biomass productivities can be obtained and contamination can be easily prevented. Yet one of the biggest problems in mass cultivation of microalgae is lack of efficient photobioreactors. Though various designs of photobioreactors have been investigated, only very few of them can utilize solar energy effectively for mass cultivation of microalgae. Most outdoor photobioreactors are characterized by large exposed illumination surfaces. There are several types of photobioreactors available, such as tubular, flat plate and column photobioreactors. Table I shows the advantages and limitations of these photobioreactors [15]. Reference [16] suggested that vertical tubular-type photobioreactors, such as bubble and air-lift photobioreactors, have always been assumed to produce the most efficient mixing, good light utilisation and the best volumetric gas transfer. These are criterias that need to be considered in a high density mass cultivation of microalgae in a photobioreactor. The air lift system produces good mixing within the photobioreactors which could improve light utilization, providing the flash light effect of microalgal photosynthesis [17]. From this point of view, tubular photobioreactors is promising except that it is limited by the high oxygen hold up within the system. A flat-plate photobioreactor has low oxygen build-up, as well as good for outdoor cultivation, good light path, high biomass productivity, and large illumination surface area. However, it is difficult to scale up this design. On the other hand, photobioreactors such as bubble-column, airlift, and stirred tank have good scalability. However, they have low illumination surfaces area which limits the efficiency of outdoor photobioreactors [18]. Reference [19] recommended the vertical flat plate photobioreactor because of its low oxygen hold up compared to tubular photobioreactor and it has high illumination area compared to column photobioreactor. The advantages of an air lift system would assist in reduction of the fouling as the cultivation media would be in constant flow and mixing would be encouraged by the bubbles. The scalability of the flat plate photobioreactor is a big limitation due to the construction material. It is costly to hold up a large volume of water in a flat plate using thick glass material. Whereas some material like polymethyl methyl acrylate PMMA deteriorates under constant exposure to outdoors conditions. The type of material used for the photo-stage is very important for an ideal photobioreactor construction. Materials such as plastic or glass sheets, collapsible or rigid tubes, with low toxicity, have high transparency, high mechanical strength, high durability, chemical stability and low cost [2] are the most suitable for microalgae cultivation.

TABLE I
ADVANTAGES AND LIMITATIONS OF PHOTOBIOREACTORS

Production system	Advantages	Limitations
Tubular photobioreactor	Large illumination surface area	Some degree of wall growth
	Suitable for outdoor cultures	Fouling
	Relatively cheap	Requires large land space
	Good biomass productivities	Gradients of pH, dissolved oxygen and CO_2 along the tubes
Flat plate photobioreactor	High biomass productivities	Difficult scale-up
	Easy to sterilise	Difficult temperature control
	Low oxygen build-up	Small degree of hydrodynamic stress
	Readily tempered	Some degree of wall growth
	Good light path	
	Large illumination surface area	
	Suitable for outdoor cultures	
Column Photobioreactor	Compact	Small illumination area
	High mass transfer	Expensive compared to open pond
	Low energy consumption	Shear stress
	Good mixing with low shear stress	Sophisticated construction

The ease of cleaning and reduction of the light transmittance after outdoor exposure are practical issues to consider. The use of fiberglass was proposed as the photobioreactor construction material. Fiberglass is made from plastic and glass fibers. It has high mechanical strength, easily molded, easily cleaned, robust, high durability, chemically stable, less brittle than glass and low cost. It is interesting to note that the light transmittance is low (i.e. 83.6%) compared to glass (95%) or polymethyl methyl acrylate PMMA (92%). Presently the effect of outdoor exposure on light transmittance reduction is unknowned for fiberglass.

The main objective of this research is to design a pilot scale of flat plate airlift-photobioreactor FP-ALPBR for mass cultivation of microalgae. Results obtained will be compared to the previous work by [1].

II. MATERIALS AND METHODS

A. Microbial Strain

The local strain of *Chlorella sp.* was obtained from Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah was used for cultivation in this research. *Chlorella sp.* was chosen because it was a local specias, thermophilic, robust, fast growing, thick cell walls (withstands high shear in a turbulent flow) and can easily adapt with outdoor tropical environment.

B. Figures

Bolds Basal Medium (BBM) was used throughout the study Table II. This medium is a widely used as an artificial freshwater medium, especially for growing green algae.

TABLE II
ADVANTAGES AND LIMITATIONS OF PHOTOBIOREACTORS

Stock Solutions		Per Liter Distilled Water (dH2O)
1.	$NaNO_3$	25.0 g
2.	$CaCl_2.2H_2O$	2.5 g
3.	$MgSO_4.7H_2O$	7.5 g
4.	K_2HPO_4	7.5 g
5.	KH_2PO_4	17.5 g
6.	NaCl	2.5 g
7.	EDTA	50.0 g
8.	KOH	31.0 g
9.	$FeSO_4.7H_2O$	4.98 g
10.	H_2SO_4	1.0 mL
11.	H_3BO_2	11.42 g
12.	Micronutrients	
	$ZnSO_4.7H_2O$	8.82 g
	$MnCl_2.4H_2O$	1.44 g
	$CuSO_4.5H_2O$	1.57 g
	$Co(NO_2)_3.6H_2O$	0.49 g

However, it is unacceptable for cultivation of many non-green algae due to lack of vitamins and some of the trace metal concentrations is relatively high [19]-[20].

C. Culture Conditions

The *Chlorella sp.* was pre-cultured in the laboratory before being inoculated into the airlift photobioreactor. Initially the pre-culture stages was done on the 20% inoculum basis (20% *Chlorella sp.* and 80% BBM). The pre-cultivation was started with a culture of 250 mL. The continuous light source was provided by means of the white fluorescence lamp and the temperature was maintained at 18±2 °C.

Next the carbon source was supplied by bubbling air into the culture and the pH level of the culture was measured using pH paper. Because of the acidifying action of CO_2 consumption, the pH level tends to decrease. Thus, NaOH solution was added to neutralize the culture.

The culture was then being scaled up to 500 mL, 1 L, 2 L, 2 L, 5 L, 15 L and 20 L autoclavable glass flasks and sterilized container before being transferred into the airlift photobioreactor. Fig. 1 shows the inoculation stages of *Chlorella sp.*

D. Materials used to Fabricate Photobioreactor

The materials for the construction of photobioreactor represent a significant practical issue both from standpoint of investment cost and performance. In this research the photobioreactor was fabricated using fiberglass.

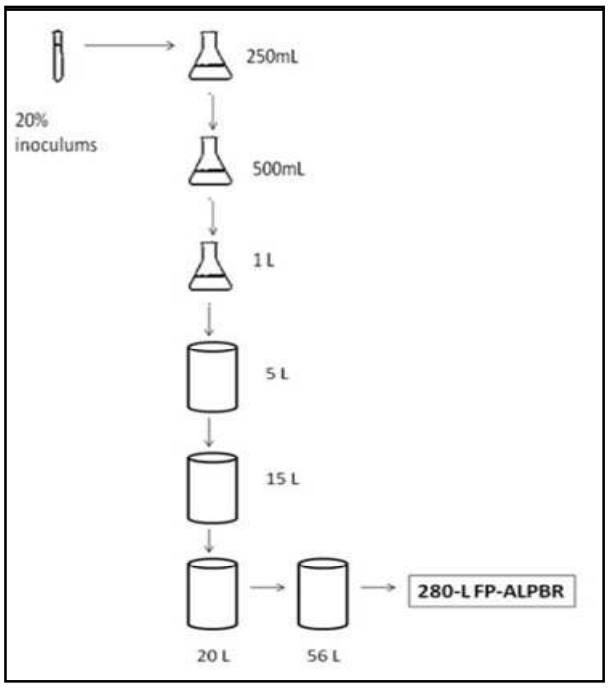

Fig. 1 Inoculation Stages

E. Photobioreactor Design

Though the design was adapted from the work of [1], there were some adjustments applied to the design of the 280 L system in this study. First was the dimension of the photobioreactor. The 280 L airlift system was constructed to the dimensions as shown in Fig. 1. The outdoor FP-ALPBR was composed of two major parts: photobioreactor body and vertical plate.

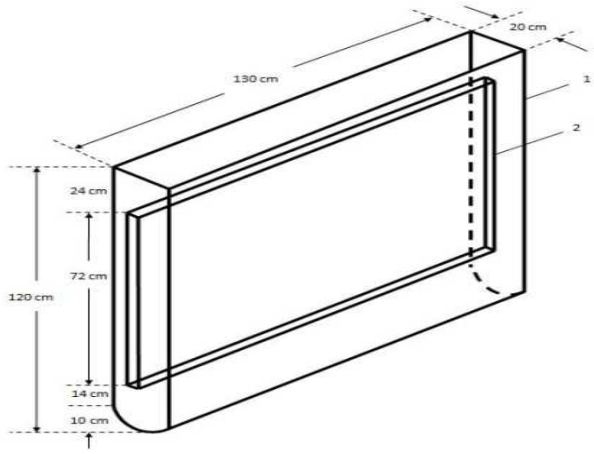

Fig. 1 Schematic representation of flat plate airlift photobioreactor (FP-ALPBR): Photobioreactor Body (1), Vertical Plate (2)

The photobioreactor body and vertical plate were made of fiber glass with thicknesses of 5 and 2 mm respectively. The column was 130 cm in length, 120 cm in height, and 20 cm in width. The total volume of the reactor was 300 L with nominal working volume of 280 L.

The vertical plate was a flat plate installed along the length of the reactor used as a separation plate. It has the same length as the photobioreactor body but different height, which was 72 cm. It was designed to separate the downcomer and the riser section. The continuous flow of the liquid culture around the vertical plate minimizes the spot dead accumulation at the base of photobioreactor body.

The vertical plate position can be varied accordingly depends on the ratio between the downcomer and riser cross-sectional areas (A_d/A_r). The downcomer cross-sectional area (A_d) was lower than the riser cross-sectional area (A_r). This maximizes the aeration efficiency, fluid flow and airlift by creating a low pressure, low density in the riser and higher liquid velocity, and thus higher pressure, in the downcomer. The pressure in the riser section was lower due to the gas bubbles released by the sparger. The difference in pressure enabled the liquid to flow from the downcomer to the riser and thus creating a circulation. The aeration was intended to keep the microalgae cells suspended as well as to promote the mass transfer (CO_2 intake and O_2 removal) inside the culture.

In this report, the ratio (A_d/A_r) was kept at 0.4, based on a previous report by (1):

$$\frac{Downriser\ \ Cross\ \ Sectional\ \ Area\ \ (A_d)}{Riser\ \ Cross\ \ Sectional\ \ Area\ \ (A_r)} \quad (1)$$

Thus

$$\frac{(5.7cm)(130cm)}{(14.1cm)(130cm)} \approx 0.4$$

The base of the photobioreactor was designed in semi cylindrical to reduce the shear stress experienced by the *Chlorella sp.* strain during the circulation in the column and to avoid dead spot. The bottom clearance was 10 cm in height to give more space for the circulation of the fluid, as well as reducing the effect of the dark region.

F. Airlift System

A gas sparger (20 mm PVC tube with 1 mm holes every 3 cm) was placed from side to side at the bottom of the reactor for aeration Fig. 2. The liquid culture in the system was agitated by passing air through the gas sparger at the bottom of the riser section.

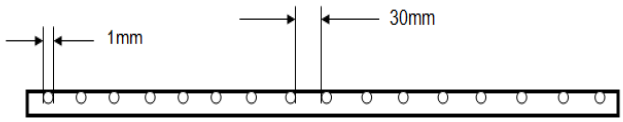

Fig. 2 Schematic representation of gas sparger

The sparger position was kept at the middle of the riser section for a better distribution of the gas bubbles. The flow rate was set as 780 $cm^3\ s^{-1}$ which gave a superficial gas velocity of 0.38 cm s^{-1}. The airlift system was tested with water before the culture was cultivated. Due to the ductility of the fiberglass (construction material) the photobioreactor expanded after being filled with water. Therefore, the height

of liquid culture must be slightly higher than the vertical plate approximate 1 cm or it will affect the shape of the photobioreactor, resulted uneven distribution of the gas bubbles.

G.Microalgae Productivity

Chlorella sp. cell density was measured microscopically twice a day (12 hours gaps) using a Neubauer hemocytometer. From the cell density, the specific growth rate (μ day^{-1}) was calculated using the following:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (2)$$

Where N_1 and N_2 (cells mL^{-1}) are cell densities at time t_1 and t_2 (days). The cell productivity (cells mL^{-1} day^{-1}) was calculated from:

$$\text{Productivity} = \frac{C_2 - C_1}{t_2 - t_1} \quad (3)$$

Where C_1 and C_2 (cells mL^{-1}) are cell density at time t_1 and t_2 (days). All the experiments were carried out in duplicate.

III. Result And Discussion

Fig. 3 shows the growth kinetics of the *Chlorella sp.* cultivated inside the 280 L system. The system gave a rather promising result with a cell productivity of 2.50×10^5 cells ml^{-1} d^{-1} and then 8.0×10^5 cells ml^{-1} d^{-1}, maximum cell density of 6.01×10^6 cells ml^{-1} and maximum growth rate of 1.47 day^{-1} and 3.04 day^{-1}. Table III shows the comparison of 17 L FP-ALPBR and 90 L FP-ALPBR conducted by [1] with the performance of the 280 L FP-ALPBR. The 280 L FP-ALPBR gave a better performance in terms of maximum cell density as well as growth rate. However, this could not compare in growth rate produced by the smaller 17 L and 90 L PB-ALPBR due to the cultivated microalgae were not the same species.

It should be noted that the 17 L and 90 L systems were cultivated under different culture condition compared to the 280 L system. As reported by [1], the 17 L and 90 L systems were run under optimum conditions (i.e. A_d/A_r=0.4, superficial gas velocity u_{sg}=0.4 cm s^{-1}, 1% CO_2 in the air supply, light intensity=20 μmol photon m^{-2} s^{-1}, and pH=7) which have been tested before the cultivation.

On the other hand, the 280 L system was run under the natural outdoor environment, the fiberglass material was opaque and did not transmit much light. Therefore the optimum conditions to grow the culture were not achieved due to the less than optimum weather conditions. T he temperature of the surroundings was between 27 ºC to 33.3ºC during day light time and this affected the cultivation of the microalgae. At the same time the culture was exposed to rain for a few days. This resulted to insufficient light intensity received by the culture. This could be responsible for the relatively low growth rate of the microalgae in the 280 L system. Yet in view of the low light transmittance of the fiberglass it is interesting to note that the cell density was a magnitude higher compared to the results given by [1].

TABLE I
RESULT COMPARASION OF 280 L WITH 90 L & 17 L.

FP-ALPBR	Maximum Cell Density	Maximum Growth Rate
17 L*	4.0 x 10^5 cells ml^{-1}	0.52 day^{-1}
90 L*	4.0 x 10^5 cells ml^{-1}	0.39 day^{-1}
280 L	6.0 x 10^6 cells ml^{-1}	1.47-3.04 day^{-1}

*Maximum cell density and maximum cell growth rate for 17 L and 90 L are from [1].

It is also of interest to note that in this study, *Chlorella sp.* has been cultivated instead of *H. pluvialis* which have been used in the previous work. The difference in terms of cultivation days might as well contribute to the different specific growth rates, but this information was not mentioned in the previous work by [1]. The microalgae inside 280 L system has only been cultivated for 20 days and have not yet reached maturity. Hence this point cannot be used to compare performances and that the elimination of specific control condition has resulted in the specific growth rate reduction. But in comparison with *Chlorella vulgaris* (see Table IV) the specific growth rate based on cell count is comparably higher than the value obtained at the same conditions. It was only outperformed by cultivation at elevated amounts of carbon dioxide.

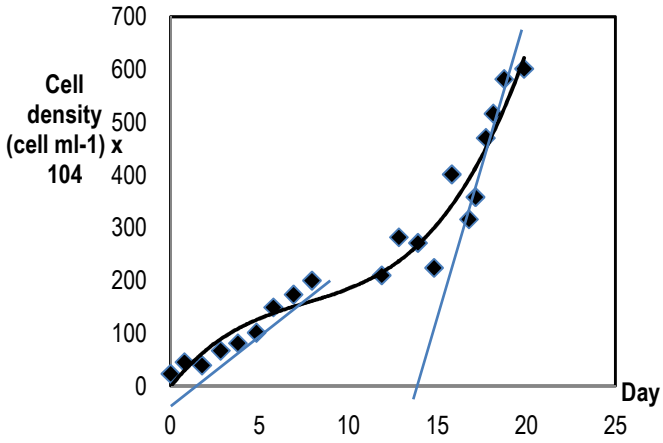

Fig. 3 Growth kinetics of *Chlorella sp.* cultivated in 280 L FP-ALPBR

IV. Conclusion

In conclusion, there is lack of efficient photobioreactors that can utilize solar energy effectively for mass cultivation of microalgae. The successful cultivation of *Chlorella sp.* in the 280 L FP-ALPBR showed that this system could be scaled up and using cheaper material (fiberglass).

TABLE IV
SPECIFIC GROWTH RATE (BASED ON BIOMASS) OF C. VULGARIS

CO_2 (%)	Temperature (ºC)	Specific growth rate based on biomass ($\mu_{biomass}$, d^{-1})
0.036 (ambient)	30	0.128
	40	0.082
	50	No Growth
6 (elevated)	30	0.222
	40	0.136
	50	0.065

Information taken from [12]

The system gave promising result with a cell productivity of 2.50×10^5 cells ml^{-1} d^{-1}, maximum cell density of 6.01×10^6 cells ml^{-1} and maximum growth rate of 1.47 day^{-1} - 3.04 day^{-1} was achieved.

ACKNOWLEDGMENT

The authors would like to acknowledge the grant no. FRG0209-TK-1/2010, MOHE, Materials and Minerals Research Unit of Universiti Malaysia Sabah for providing the materials and facilities throughout the completion of this research work.

REFERENCES

[1] K. Issarapayup, S. Powtongsook, and P. Pavasant, "Flat panel airlift photobioreactors for cultivation of vegetative cells of microalga Haematococcus pluvialis," *Journal of Biotechnology*, 2009, 142, 227-232.

[2] A. Richmond, *Handbook of microalgal culture: biotechnology and applied phycology*, Oxford : Blackwell Science Ltd., 2007. ISBN 978-0-632-05953-9.

[3] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances*, 2007 25(3), 294-306.

[4] A. B. M. S. Hossain, "Biodiesel fuel production from algae as renewable energy," *American Journal of Biochemistry and Biotechnology*, 2008, 4(3), 250-254.

[5] P. M. Schenk, C. Posten, O. Kruse and B. Hankamer, "Second generation biofuels: High-efficiency microalgae for biodiesel production," *Bioenergy Res.* 2008, 1(1), pp. 1939–1234.

[6] K. Tsukahara, and S.Sawayama, "Liquid fuel production using microalgae," *Journal of the Japan Petroleum Institute*, 2005, 48(5), 251-259.

[7] Q. Hu, "Microalgal triacylglycerols as feedstocks for biofuels production: perspectives and advances," *The Plant Journal*, 2008, 54, 621-639.

[8] L. Rodolfi, "Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor," *Biotechnology and Bioengineering*, 2009, 102(1), 100-112.

[9] J. N. Rosenberg, "A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution," *Current Opinion in Biotechnology*. 19(5), pp. 430-436.

[10] Y. Li, "Biofuels from microalgae," *Biotechnology Progress.* 2008, 24(4), pp. 815-820.

[11] K. L. Kadam, "Microalgae Production from Power Plant Flue Gas: Environmental Implications on a life cycle basis," Colorado : NREL, 2001, 63 pp, NREL/TP-51029417.

[12] S. Chinnasamy, "Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications," *Bioresour Technol*., 2010, 101 (9).

[13] C. G. Lee, and B.Ø.Palsson, "Light emitting diode-based algal photobioreactor with external gas exchange," *J. Ferment. Bioeng.*, 1995, 79, pp. 257-263.

[14] G. Cogne, J. F. Cornet and J. B. Gross, "Design, operation, and modeling of a membrane photobioreactor to study the growth of the cyanobacterium Arthrospira platensis in space conditions," *Biotechnology Progress.*, 2005, 21, pp. 741-750.

[15] L. Brennan and P. Owende, "Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products," *Renew. Sustain. Energy Rev*., 2010, 14, pp. 557–577.

[16] N. Eriksen, "The technology of microalgal culturing," *Biotechnology Letters*, 2008, 30(9), pp. 1525–1536.

[17] M. J. Barbosa, Microalgae cultivation in air-lift reactors: Modeling biomass yield and growth rate as a function of mixing frequency. *Biotechnol. Bioeng.* 2002, 82(2), pp. 170-179.

[18] C. U. Ugwu, H. Aoyagi and H. Uchiyama, C. U. Aoyagi, and H. Uchiyama, "Photbioreactors for mass cultivation of algae," *Bioresource Technology*, 2010, 99(10), pp. 4021-4028.

[19] H. C. Bold, "The morphology of Chlamydomonas chlamydogama sp.," *nov. Bull. Torrey Bot. Club.*, 1949, 76, pp. 101-108.

[20] H. W. Bischoff, and H. C. Bold, "Some soil algae from Enchanted Rock and related algal species," *Phycological Studies IV*, 1963, 6318, pp. 1-95.

[21] R. R. L. Guillard and M. S. Sieracki, "Counting Cells in Cultures with the Microscope," book auth. Robert A. Andersen. *Algal Culturing Technique*, Hong Kong : Elsevier, 2005, pp. pg 239-252.

[22] M. H. Lu. *Design of a Pilot Scale Outdoor Photobioreactor for Mass Cultivation of Microalgae*, A Thesis for a BEng Final Year Project. Kota Kinabalu : University Malaysia Sabah, 2011.

[23] G. E. Fogg and B. Thake, *Algal cultures and phytoplankton ecology*. s.l. : University of Wisconsin Press, 1987.

[24] B. L. Björn, *Photobiology: the science of light and life*. Sweeden : Springer, 2002 .

[25] C. N. Dasgupta, Recent trends on the development of photobiological. *International journal of hydrogen energy*. Article in Press, 2010.

[26] M. R. Tredici, Bioreactors, photo. *Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation*. New york : Wiley, 1999. Vol. 1, p. 395-419.

[27] A. Demirbas, "Use of Algae as Biofuel Sources. Energy Conversion and Management," 2010, Vol. 51, pp. 2738-2749.

[28] H. C. Greenwell, L. M. L. Laurens, R. J. Shields, R. W. Lovitt, and K. J. Flynn, "Placing microalgae on the biofuels priority list: a review of the technology challenges," *Journal of the Royal Society Interface the Royal Society*, 2010, Vol. 7, pp. 703-726.

[29] O. Pulz, "Photobioreactors: production systems for phototrophic microorganisms," *Applied Microbiology and Biotechnology*. 2001, 57(3), pp. 287–293.

[30] M. Olaizola and M. E. Huntley, "Recent advances in commercial production of astaxanthin from microalgae," In M. Fingerman and R. Nagabhushman. *Biomaterials and bioprocessing*, Enfield Science Publ., 2003, pp. 143-164.

[31] M. A. Borowitzka, "Commercial production of microalgae: ponds, tanks,tubes and fermenters," *Journal of Biotechnology*. 1999, 70(1-3), pp. 313-321.

[32] T. I. Zebib, "Microalgae Grown in Photobioreactors for Mass Production of Biofuel," *Department of Bioenvironmental Engineering*. 2008.