

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Flashing Light as Growth Stimulant in Cultivation of Green Microalgae, *Chlorella* sp. Utilizing Airlift Photobioreactor

<sup>1,2</sup>L. Fathurrahman, <sup>1</sup>A.H. Siti Hajar, <sup>1</sup>D. Wan Nur Sakinah, <sup>1</sup>Z. Nurhazwani and <sup>1,2</sup>J. Ahmad  
<sup>1</sup>Department of Engineering Science, Faculty of Science and Technology,  
Universiti Malaysia Terengganu, Jln. Tok Jembal, 21030, Terengganu, Malaysia  
<sup>2</sup>Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu,  
Jln. Tok Jembal, 21030, Terengganu, Malaysia

**Abstract:** One of the main limitations of productivity in photobioreactor is the inefficient conversion of the available light into biomass. Photoautotrophic cells such as microalgae only absorb a small fraction of supplied illumination due to limitation of its photosystem's (PS) absorbing rate. However, phenomenon of Flashing Light Effect (FLE) allows microalgae to utilize strong light exceptionally through intermittent exposure. Exposure of strong light at correct frequency of light and dark photoperiod would allow two pigment-protein complexes, PSI and PSII to be at the equilibrium mid-point potential to allow efficient light conversion. Narrow range of optimum frequency is crucial since overexposure to strong light would injured photosynthetic apparatus whereas longer dark period would contributed to loss of biomass due to triacylglycerol metabolism. The behaviour of microalgae towards various illumination conditions of FLE was determined at batch Photobioreactor (PBR) by varying the aeration flow rate: 16.94, 33.14 and 49.28 mL sec<sup>-1</sup> which yield, respectively the light exposure time of 3.99, 1.71 and 1.1 seconds per cycle. Maximum cell density in FLE-PBR was significantly higher at the exponential phase as compared to the continuously illuminated culture ( $p = 5.62 \times 10^{-5}$ ,  $\alpha = 0.05$ ) under the flow rate of 25.07 mL sec<sup>-1</sup>. Maximum cell density yield of FLE-PBR and continuously illuminated PBR was, respectively  $3.1125 \times 10^7$  and  $2.947 \times 10^7$  cells mL<sup>-1</sup>. Utilization of FLE as an innovative solution to increase the efficiency of microalgae to convert light into chemical energy would revolutionize the microalgae culture, reduce the time for cultivation and produce higher maximum biomass density.

**Key words:** *Chlorella* sp., growth stimulant, flashing light effect, intermittent illumination

### INTRODUCTION

Microalgae produce many useful and beneficial compounds for the sustainability of the ecosystem. Microalgae biomass is widely used as human food and aquaculture feed. Microalgae biotechnology is increasingly recognized as one of the emerging fields in the agriculture and aquaculture industries. In addition, microalgae culture could also potentially provide a way of reducing the amount of accumulated carbon dioxide in the atmosphere (Demirbas, 2011a; Demirbas, 2010; Suali and Sarbatly, 2012). Recently, there has been a concerted interest in using microalgae as an innovative and environmentally friendly source for production of oils for renewable energy and pharmaceutical purposes (Demirbas, 2011b; Ilavarasi *et al.*, 2011). Due to their capability to produce structurally complex compounds, microalgae could potentially serve as an important natural source of bioactive molecules (Ming *et al.*, 2012).

Mass cultivation of microalgae required the utilization of photobioreactors for completely-controlled cultivation and large scale production. Until today, a variety of photobioreactors have been proposed and developed for mass microalgae cultivation (Richmond, 2008). However, these are either too complex or too costly to be applied in large-scale production. Hence, the utilization of photobioreactors are considered as capital-intensive approaches due to the high development cost and they would only justified when a fine and valuable chemical is to be produced (Brennan and Owende, 2010; De La Noue and De Pauw, 1988). However, the rapidly growing aquaculture industry had created a growing demand for microalgae as live feed source for larval feeding (Richmond, 2008). Hence, the challenge faced worldwide is to reduce the construction costs of photobioreactor systems to make them more economically competitive and viable for adaptation for the mass production. There are several factors contributing to the low productivity of

microalgae. Some of them are photo-inhibition in the intensely illuminated outer zones of photobioreactor, inefficient conversion of the available light into the cell biomass, build-up of excess inhibitory oxygen, depletion of biomass due to respiration in the photobioreactor's dark zones and inadequate mixing of CO<sub>2</sub> and nutrients (Degen *et al.*, 2001; Sato *et al.*, 2010).

Burlew (1953) stated that via the intermittent exposure, photoautotrophic cells such as microalgae will have an improved utilization of strong illumination. This approach could be adapted to increase the conversion of light energy into biomass by allowing the microalgae cell to be repeatedly exposed to the condition of strong light and darkness. This type of energy conversion enhancement is known as the light-dark cycle or FLE (Kok, 1956; Terry, 1986).

Several designs of photobioreactors have been reported to attempt the application of FLE for microalgae productivity enhancement (Degen *et al.*, 2001; Sato *et al.*, 2010; Xu *et al.*, 2002; Yoshimoto *et al.*, 2005). However, a regular mixing pattern has not generally been implemented. Due to that, this study had focus on a new photobioreactor that enhances productivity by effectively utilizing the flashing-light effect and regular mixing channel. This study emphasized on the engineering of a simple, low-cost, yet efficient airlift photobioreactor. Cylinder body will be used to provide wide illumination surface area and a draft tube to induce a regular light cycling and flow of microalgae. Commonly, there are two basic approaches for exposing the cells to intermittent illumination: the first method entails the use of a light source or a system which provides illumination intermittently (Richmond, 2008). The application of flashing light source may only be acceptable for low microalgae cell densities, in which mutual shading is essentially absent, thus it is being useless for mass cultures and whenever high productivity is expected. The second probability is the only practical by using a continuous light source whether in the lab or outdoors and has the cells move at a high frequency, in and out of the illuminated volume. The second approach was selected to be utilized in this study.

This study was performed based on the objectives to determine the effect of FLE on the growth performance of *Chlorella* sp. and the optimum aeration flow rate to induce FLE for the maximum growth yield of *Chlorella* sp. FLE system was design to provide two types of illumination areas: the dark region and illuminated region. The illuminated cells (at the illuminated region), which are replaced by dark cells, were shifted to the dark volume while these former dark cells are in their turn, illuminated. In this manner, more microalgae cells such as

in dense cultures are exposed to light flashes per unit of time. Strong light which is higher by an order of magnitude than saturating light, is in effect diluted by being available in smaller doses to more microalgae cells along a given time span, thus being used more efficiently as compared to the light utilization of microalgae cells illuminated continuously in low density or poorly stirred cultures.

## MATERIALS AND METHODS

**Cultivation of *Chlorella* sp.:** *Chlorella* sp. was obtained from stock culture collection in Live Feed Culture Laboratory of Institute of Tropical Aquaculture, UMT. *Chlorella* sp. was obtained in the form 10 mL monoculture which then up-scaled and maintained in 1000 mL bottle. Conway medium based on Tompkins *et al.* (1995) were used as growth nutrient were added into 10 mL of inoculum and it was kept under illumination in an air-conditioned room at temperature of 22±2°C. Cell count was performed using a Haemocytometer technique coupled with light absorption at 678 nm via Shimadzu UV1800 Dual-Beam Spectrophotometer.

**Specification of the airlift photobioreactor:** The photobioreactor is consists of three major parts: outer tube, draft tube and air duct. The outer tube will provide an illumination surface area of 8.67×10<sup>-2</sup> m<sup>2</sup>. A concentric draft tube also called riser was located coaxially within the outer tube. Four opening holes with the total area of 6×10<sup>-4</sup> m<sup>2</sup> were constructed in order to allow liquid circulate through the riser and down-comer. By joining the circular cover, an air duct was located coaxially inside the draft tube. The photobioreactor has working volume of 4×10<sup>-3</sup> m<sup>3</sup>; outer tube internal diameter 0.13 m; outer tube height 0.34 m; draft tube internal diameter 9.2×10<sup>-1</sup> m; draft tube height 0.37 m; air duct internal diameter 5×10<sup>-3</sup> m.

Design of the bioreactor was based on the study of (Xu *et al.*, 2002). Modification was done by introducing opaque draft tube as replacement to transparent tube in order to create FLE (Fig. 1). In this study, three illumination conditions were introduced: intermittence, continuous illumination and unlighted condition. Each illumination variables was performed in triplicates. Both microalgae growth performance and aeration flow rate of each replicates were recorded and analysed. Bright culture condition (positive control) was established by removing the draft tube from the PBR system. Dark culture condition (negative control) established with the same manner by wrapping aluminium foils on the overall external structure of the PBR system.

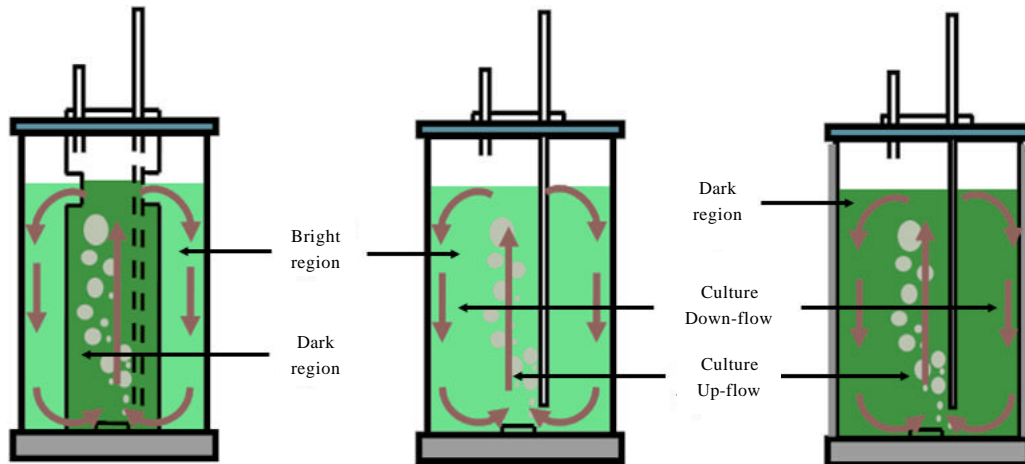


Fig. 1: Schematic diagram of the operating condition of the PBR system under FLE condition (left), continuously illuminated condition (middle) and unlighted condition (right). For FLE-PBR, culture will move in designated speed alternatively to the dark region and the illuminated area during its operation. Continuously illuminated PBR had no opaque draught tube which exposed microalgae culture to illumination whereas unlighted condition PBR had its external cylinder body coated with opaque black paint

**Aeration flow rate variables:** Aeration flow rate was regulated by varying the flow rate: low, intermediate and high. Low flow rate was established by observing the lowest possible aeration flow rate required to allow a complete liquid circulation from inside draught tube to the illumination area. On the contrary, high flow rate was introduced by completely opening the aeration valve allowing as maximum aeration as possible into the PBR system. Intermediate flow rate was done by regulating the aeration at mid-point valve opening between the low and high flow rate specified.

Volumetric flow rate measurement based on Recktenwald (2006) was used to quantify each specified aeration flow rate. Ten replicates were carried out for each flow rate before inoculation of microalgae performed. Regular monitor of the aeration flow rate was performed to ensure uniform flow rate throughout the culture period. As shown in Fig. 2, the low, intermediate and high flow rates was predetermined at 16.94, 33.14 and 49.28 mL sec<sup>-1</sup>, respectively prior to inoculation of microalgae biomass into each photobioreactor.

**Liquid circulation velocity:** A tracer method was employed for the measurement of liquid circulation velocity in the airlift reactor (Chisti, 1989). A small amount of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the reactor to adjust pH to about 3 and the reactor was bubbled with air (superficial gas velocity in the riser, U<sub>GR</sub> ~ 40 mm sec<sup>-1</sup>) for about 2 h. When the liquid showed no buffering over the pH range (pH = 4) of the measurements and 200 mL of

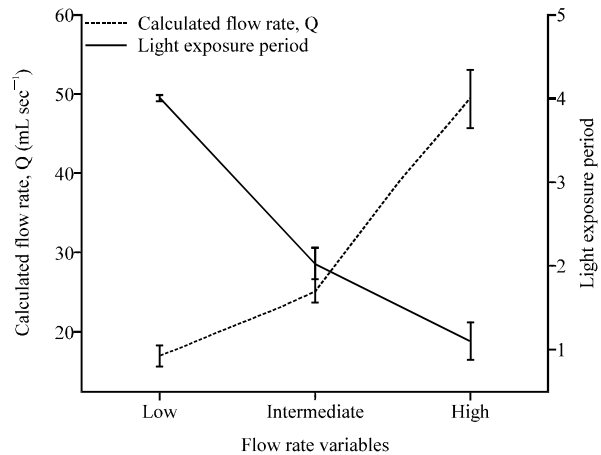


Fig. 2: Calculated aeration flow rate and its respective light exposure period at different flow rates

8 M H<sub>2</sub>SO<sub>4</sub> was poured instantaneously into the top of the down-comer.

The linear liquid velocity ( $V_{Ld}$ ) in the down-comer was determined by dividing the vertical distance (0.3 m) between the tracer peaks of the two pH electrodes with the corresponding time taken according to Miron *et al.* (1999).  $V_{Ld}$  is represented the superficial velocity ( $U_{Lr}$ ) in the riser based in the continuity relationship by Chisti (1989):  $U_{Lr}A_r = V_{Lr}A_r(1-\epsilon_r) = (V_{Ld}A_d(1-\epsilon_d)) = U_{Ld}A_d$ , where  $V_{Lr}$  is the linear liquid velocity in the down-comer,  $A_r$  and  $A_d$  are cross-sectional area of the riser and the down-comer, respectively. The mean circulation time

( $t_c$ ) in the airlift reactor was defined by the sum of the residence time in the riser ( $t_r$ ) and in the down-comer ( $t_d$ ) and computed using analytical relationships (Chisti, 1989):  $t_c = t_r + t_d = L_r/U_{Lr} + L_d/U_{Ld}$ , where  $L_r$  and  $L_d$  are the length of the riser and the down-comer, respectively. As shown in Fig. 2, light exposure period per liquid circulation regarding three predetermined aeration period is 3.99 sec for low, 1.71 sec for intermediate and 1.1 sec for high aeration flow rate.

**Microalgae growth parameter:** Sampling of biomass was performed daily until microalgae reached their death phase for about 12-14 days. Aliquot of 2 mL was taken and placed on haemocytometer for cell density determination under light microscope. Observation was performed using National Optical (tm) microscope coupled with MOTIC DMC-300 VGA camera for digital visualization. Absorbance of biomass was determined at 678 nm as reference on calculated cell density. A determination of cell density was computed using Microsoft Office Excel™ in order to form growth curve.

Numerical and graphical methods were employed to determine the significance between both variables, i.e., flow rate and illumination condition. Maximum cell production and net cell production throughout 14-days cultivation period was performed using area under curve analysis by integrating the polynomial equation of the growth curve. One-way ANOVA with 95% confidence interval was utilized for comparison on the growth performance using Minitab 16™.

## RESULTS

**Batch trial and establishment of photo-inhibition condition:** Microalgae cell density was recorded to form mean growth evolution curve and the data was taken from six replications performed under photo-inhibition condition. Figure 3 shows growth evolution curve depicting a normal growth phase compared to growth evolution under photo-inhibition condition. Cell growth under photo-inhibition had suppressed growth, which was lower order exponential growth phase as compared to cell growth under normal illumination. Illumination was evaluated for the capacity to induce photo-inhibition in the microalgae population. Photo-inhibition determination was done to ensure supplied illumination was at least at an order higher than the light saturation value. Illumination capable of inducing photo-inhibition was important in order to simulate the effect of FLE (Burlaw, 1953; Kok, 1956; Richmond, 2008; Terry, 1986; Yoshimoto *et al.*, 2005). Culturing condition under normal illumination which was light below saturation point was

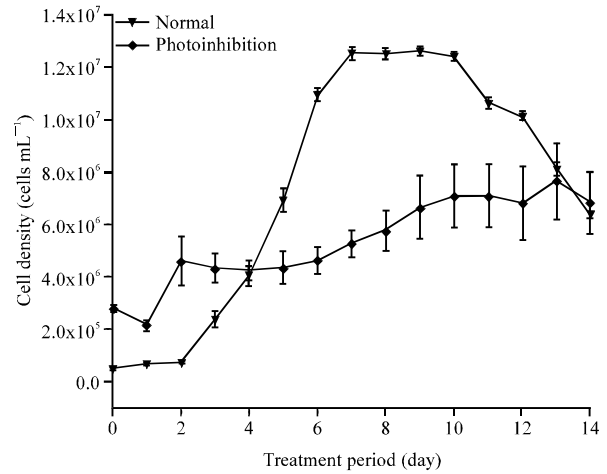


Fig. 3: Microalgae growth evolutions under saturating illumination as compared to normal illumination throughout 14 days treatment period

performed in order to compare microalgae growth evolution to the illumination and growth under photo-inhibition.

**Microalgae growth performance under designated FLE-Photobioreactor:** Effects of FLE were measured at three different flow rate conditions; low, intermediate and high flow rate. As shown in Fig. 4, mean growth evolution of FLE, continuously illuminated culture condition and unlighted culture condition throughout 14 days period were plotted. At low flow rate, continuously illuminated culture condition and FLE growth performance in terms of maximum cell density, maximum cell production and net cell production was not significantly different from each other. FLE had lower order of growth at exponential phase and short sustain at the stationary phase compared to bright culture condition. In this condition, growth performance of microalgae at continuous illumination culture condition was better than FLE culture condition. Low aeration flow rate caused low liquid circulation velocity. At low liquid circulation velocity, alternating frequency of light flash would also become lower. However, lower frequency of light flash inversely proportional to light exposure period. At low flow rate, light exposure period recorded was  $3.99 \pm 0.037$  sec. But, higher light exposure period per liquid circulation contributed to higher dark period since each cycle divided into two region only-light exposure and dark period.

At intermediate flow rate, differences between continuous illumination and FLE culture condition could be observed at exponential and stationary phase of microalgae growth. FLE had higher order of exponential

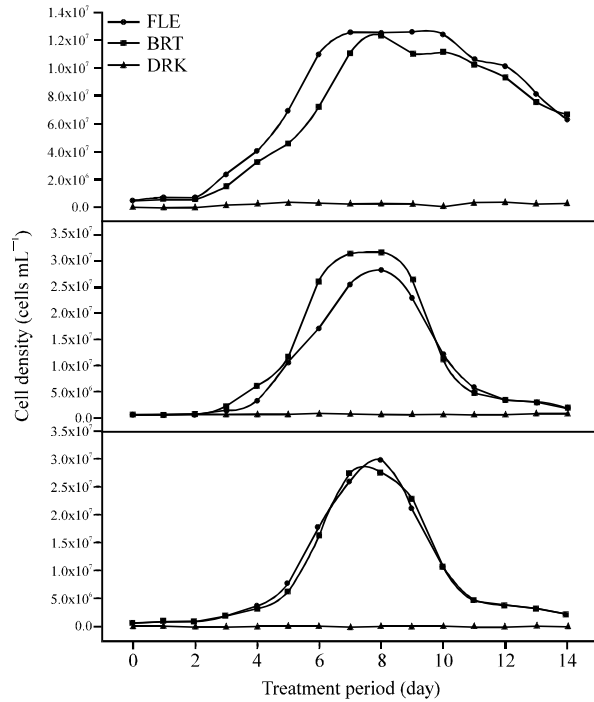


Fig. 4: Microalgae growth evolutions at three illumination conditions-FLE, bright condition and dark condition at low flow rate (top), intermediate flow rate (middle) and high flow rate (bottom) throughout 14 days treatment period

growth and higher maximal cell density reading as compared to continuous illumination culture condition. No clear observable differences recorded at the death phase of both culture conditions. Under intermediate flow rate, mean light exposure period recorded was  $2.02 \pm 0.19$  sec. Microalgae culture was alternating between the illuminated and dark condition at higher velocity thus reducing the dark period. This would contribute to lower respiration and triacylglycerol metabolism for cell division. However, the reduction would be at the right balance-not too low and not too high-that metabolized triacylglycerol in the dark has been compensated with its synthesis at the illuminated area.

Lastly, for the high flow rate, no clear differences observed on the pattern of growth evolution between FLE and bright culture condition throughout the culturing period. At high flow rate, microalgae culture was subjected to high Reynolds which is the degree of vigorous mixing allowing sufficient contact between microalgae cells and nutrient molecules inside the media. High flow rate would also contribute to higher liquid circulation velocity which caused the culture to pass through illuminated area in short time. Recorded light exposure period for this flow rate was  $1.1 \pm 0.22$  sec.

**Maximum cell density, cell production and treatment period between culture conditions:**

Maximum cell density is defined as the given concentration of microalgae biomass at certain time whereas maximum cell production is the cumulative number of microalgae biomass concentration from inoculation to the day when maximum cell production is achieved. In addition, net cell production is the total cumulative number of microalgae biomass concentration from inoculation to the 14 days period. As shown in Table 1, FLE had the highest maximum cell density as compared to other culture conditions. However, FLE and continuously illuminated culture at both the intermediate and high flow rate still not significantly different at 95% confidence interval. In the contrary, maximum cell production for FLE was significantly higher as compared to other illumination conditions.

**DISCUSSION**

The study for batch and establishment of photo-inhibition focused on the growth rate of cells and conducted in two conditions; photo-inhibition and normal illumination. Under the condition of photo-inhibition, microalgae growth especially at the exponential phase or log phase was suppressed as compared to the growth under normal illumination. Under normal lighting, the supplied amount of illumination was either at the same level or lesser than those could be consumed by the photon reaction centre. According to Degen *et al.* (2001), *Chlorella* sp. has the ability to absorb more photon than what it required and this protect the cell from photo-damage. Richmond (2008) supported that the species of plants would exhibit the same growth phase, whether they are from lower or higher type, under optimum range of illumination, nutrition and temperature and ambient water condition. Velikova *et al.* (2000) showed that photo-inhibition has been related to PS II part of photosynthetic electron transport chain. Strong illumination could at some level contributed to the decrease in photosynthetic activity. Once absorbed light energy reached reaction centre which exceeds its consumption, the photosynthetic apparatus can be injured (Demmig-Adams and Adams, 1996; Ruban *et al.*, 1994). Under condition of normal illumination, all of the growth phases were clearly depicted on the plotted growth evolution curve.

Microalgae growth performance under designated FLE was measured at three different flow rate conditions. For the low flow rate study, Degen *et al.* (2001) reported consumption of biomass through respiration happen in the dark zone of bioreactor. This is supported by Sukenik *et al.* (1989) stating that dark condition plays role

Table 1: Microalgae growth parameters at different flow rates and illumination conditions. Statistically different means within each category with 95% confidence interval is grouped in subsets represented with superscript

PBR Condition	Maximum cell density(cells mL <sup>-1</sup> )	Time to peak(day)	Maximum cell production (cells)	Time at max. cell production (day)	Net cell production throughout 14-days cultivation (cells)
Low flow rate (FLE)	12,348,888.89±390,801 <sup>b</sup>	8.13±0.31 <sup>a</sup>	11,513,920.69±68,971.13 <sup>c</sup>	8.50±1.00 <sup>f</sup>	6,585,259.32±211,028.95 <sup>a</sup>
Low flow rate (Cont. illumination)	12,735,555.56±55,511 <sup>b</sup>	8.22±0.28 <sup>a</sup>	11,980,950.17±50,456.71 <sup>c</sup>	9.30±0.61 <sup>b,c</sup>	6,620,241.11±27,880.56 <sup>a</sup>
Low flow rate (Unlighted)	988,888.89±60,491 <sup>e</sup>	10.37±0.74 <sup>a</sup>	333,577.70±18,740.40 <sup>d</sup>	12.23±1.01 <sup>a</sup>	241,345.50±13,558.79 <sup>f</sup>
Intermediate flow rate (FLE)	32,100,000.00±458,160 <sup>a</sup>	7.69±0.20 <sup>a</sup>	30,823,875.02±301,375.19 <sup>a</sup>	8.13±1.00 <sup>f</sup>	1,820,835.83±17,802.91 <sup>b</sup>
Intermediate flow rate (Cont. illumination)	28,848,888.89±1,601,337 <sup>a</sup>	7.67±0.58 <sup>a</sup>	26,827,185.87±1,362,801.36 <sup>a</sup>	8.40 ±0.87 <sup>e</sup>	2,049,110.53±104,093.31 <sup>b</sup>
Intermediate flow rate (Unlighted)	866,666.67±80,829 <sup>e</sup>	8.67±3.79 <sup>a</sup>	285,511.95±23,458.98 <sup>d</sup>	8.63±0.13 <sup>e</sup>	285,511.95±23,458.98 <sup>f</sup>
High flow rate (FLE)	28,804,444.44±3,209,128 <sup>a</sup>	7.67±0.58 <sup>a</sup>	26,297,713.10±2,160,741.91 <sup>b</sup>	7.68±0.29 <sup>f</sup>	1,903,783.93±156,423.71 <sup>b</sup>
High flow rate (Cont. illumination)	29,795,555.56±2,367,280 <sup>a</sup>	7.67±0.58 <sup>a</sup>	27,229,181.73±2,237,275.69 <sup>b</sup>	8.10±0.26 <sup>f</sup>	2,050,897.46±168,511.23 <sup>b</sup>
High flow rate (Unlighted)	857,777.78±49,140 <sup>e</sup>	11.33±4.62 <sup>a</sup>	282.46±23.21 <sup>d</sup>	11.32±0.56 <sup>b,b</sup>	282.46±23.21 <sup>e</sup>

in photosynthesis and growth since triacylglycerol is metabolized rapidly only in the dark. Long period of darkness inside FLE culture condition under this particular flow rate would contribute to the loss of microalgae biomass thus suppressing its growth especially at the exponential phase. As compared to intermediate and high flow rate, both FLE and continuous illumination culture condition at low flow rate had very low maximal cell density recorded at the stationary phase. So, it could be presumed that supplied flow rate was too slow to supply optimal mixing in addition to the long dark photoperiod. According to Richmond (2008), sufficient mixing rate, quantify by the Reynolds number, at certain minimum level is required in order to allow optimum cell growth. Flow rate that was too slow would also not sufficient to prevent the formation of biofilm which could be observed at almost PBR of this particular flow rate.

Under intermediated flow rate, growth performance under FLE culture condition was observed to be better as compared to bright culture condition. Maximal cell density yield and exponential growth order of FLE was observed to be higher than the bright culture condition. Illumination used for the culturing condition was considered strong light since it contributed to the photo-inhibition of batch culture test. In order to utilize strong light efficiently for growth, intermittent manner of illumination is required (Kok, 1956; Sato *et al.*, 2010; Sukenik *et al.* 1989). Allowing culture media to flow between dark and illuminated area would contribute to the effect of light dilution and also provide adequate region for microalgae cell division. Lastly, under high flow rate condition, shorter light exposure period leads to higher alternating frequency. At this degree, the alternating frequency was very high until it reached the level where dark photoperiod almost negligible approaching the characteristics of bright culture condition. Thus, this explained why the difference between FLE culture condition and bright culture condition was insignificant.

Taking FLE-PBR as constant variable in term of physical aspects in bioreactor-mixing pattern, volume, growth media and illumination area-the effect between varieties of flow rate introduced could be observed. One-way ANOVA was selected to test the effect of different flow rate on the growth of microalgae inside the FLE-PBR system. Significance within replicates between different flow rates had been omitted to take into consideration the possibility of natural variation. This comparison would depict the best aeration flow rate within and limited to the engineered PBR system itself.

### CONCLUSION

Effects of flashing light on the growth of *Chlorella* sp. in a bioreactor system was elucidated successfully in this study. Intermediate flow rate of 25.07 mL sec<sup>-1</sup> has been determined as the most optimum flow rate as compared to the other three flow rates. Low flow rate was insufficient to induce optimum Reynolds number for mixing and agitation of available nutrient and microalgae biomass. Increase of flow rate did not necessarily contribute to the increase in microalgae growth since it started to approach the characteristics of continuous illumination at high level of liquid circulation velocity. Maximal cell density of 3.113×10<sup>6</sup> cells mL<sup>-1</sup> was recorded at the intermediate flow rate which is higher than both low and high aeration flow rate.

### ACKNOWLEDGMENT

The authors would like to thank the laboratory staff of Institute of Tropical Aquaculture at Universiti Malaysia Terengganu for their assistance during this study. This project was financially funded by the Institute of Tropical Aquaculture's Master in Science Course work Structure Programme.

## REFERENCES

- Brennan, L. and P. Owende, 2010. Biofuels from microalgae-A review of technologies for production, processing and extractions of biofuels and co-products. *Renewable Sustainable Energy Rev.*, 14: 557-577.
- Burlew, J.S., 1953. *Algae Culture: From Laboratory to Pilot Plant*. Carnegie Institution of Washington, Washington, DC., pp: 357.
- Chisti, M.Y., 1989. *Airlift Bioreactors*. Elsevier Applied Science, London, UK., ISBN-13: 9781851663200, Pages: 345.
- De La Noue, J. and N. De Pauw, 1988. The potential of microalgal biotechnology: A review of production and uses of microalgae. *Biotechnol. Adv.*, 6: 725-770.
- Degen, J., A. Uebele, A. Retze, U. Schmid-Staiger and W. Trosch, 2001. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *J. Biotechnol.*, 92: 89-94.
- Demirbas, A., 2011a. Biodiesel from oilgae, biofixation of carbon dioxide by microalgae: A solution to pollution problems. *Applied Energy*, 88: 3541-3547.
- Demirbas, A., 2011b. Competitive liquid biofuels from biomass. *Appl. Energy*, 88: 17-28.
- Demirbas, F.M., 2010. Microalgae as a feedstock for biodiesel. *Energy Educ. Sci. Technol. A*, 25: 31-43.
- Demmig-Adams, B. and W.W. Adams, 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci.*, 1: 21-26.
- Ilavarasi, A., D. Mubarakali, R. Praveenkumar, E. Baldev and N. Thajuddin, 2011. Optimization of various growth media to freshwater microalgae for biomass production. *Biotechnology*, 10: 540-545.
- Kok, B., 1956. Photosynthesis in flashing light. *Biochim. Biophys. Acta*, 21: 245-258.
- Ming, L.C., R. Nurliyana, A.B. Syah, M.N. Azizah, H.L. Sim and M.Y. Hirzun, 2012. Identification and biochemical composition of a green microalgae. *Asian J. Biotechnol.*, 4: 38-45.
- Miron, A.S., A.C. Gomez, F.G. Camacho, E.M. Grima and Y. Chisti, 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *J. Biotechnol.*, 70: 249-270.
- Recktenwald, G., 2006. Volumetric flow rate measurement. Mechanical Engineering Report, Portland State University, Portland, USA.
- Richmond, A., 2008. *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. John Wiley & Sons, New York, USA., ISBN-13: 9781405172493, Pages: 584.
- Ruban, A.V., A. Young and P. Horton, 1994. Modulation of chlorophyll fluorescence quenching in isolated light harvesting complex of Photosystem II. *Biochimica Biophysica Acta Bioenergetics*, 1186: 123-127.
- Sato, T., D. Yamada and S. Hirabayashi, 2010. Development of virtual photobioreactor for microalgae culture considering turbulent flow and flashing light effect. *Energy Conver. Manage.*, 51: 1196-1201.
- Suali, E. and R. Sarbatly, 2012. Conversion of microalgae to biofuel. *Renew. Sustain. Energy Rev.*, 16: 4316-4342.
- Sukenik, A., Y. Carmeli and T. Berner, 1989. Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. *J. Phycol.*, 25: 686-692.
- Terry, K.L., 1986. Photosynthesis in modulated light: Quantitative dependence of photosynthetic enhancement on flashing rate. *Biotechnol. Bioeng.*, 28: 988-995.
- Tompkins, J., M.M. Deville, J.G. Day and M.F. Turner, 1995. *Culture Collection of Algae and Protozoa. Catalogue of Strains*. Ambleside, UK., pp: 204.
- Velikova, V., I. Yordavov and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.*, 151: 59-66.
- Xu, Z., Z. Baicheng, Z. Yiping, C. Zhaoling, C. Wei and O. Fan, 2002. A simple and low-cost airlift photobioreactor for microalgal mass culture. *Biotechnol. Lett.*, 24: 1767-1771.
- Yoshimoto, N., T. Sato and Y. Kondo, 2005. Dynamic discrete model of flashing light effect in photosynthesis of microalgae. *J. Applied Phycol.*, 17: 207-214.