Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Development of simple floating photobioreactor design for mass culture of *Arthrospira platensis* in outdoor conditions: Effects of simple mixing variation

Mohamed Amar Naqqiuddin[a], Norsalwani Muhamad Nor[a], Hishamuddin Omar[a] & Ahmad Ismail[a,*]

[a]Biology Department, Faculty of Science, University Putra Malaysia 43400, Serdang, Selangor.

*Correspondent author, Email: *aismail@science.upm.edu.my*

Abstract

A common perception of photobioreactor is an enclosed system which allows light to penetrate through with suitable circulation, cooling system and perfected with arrays of sensor to monitor culture parameters. This paper presents simple floating photobioractor (PBR) experiments that were placed on water bodies without any facilities of computerized controlled systems. The idea is to study the effects of different photobioreactors shape and different aeration placement on the productivity of *Arthrospira platensis* (Spirulina). In this study, simple floating PBRs were designed in two different shape form using water container, Polyethylene terephthalate (PET) materials. Simple land PBR was prepared with High-density Polyethylene (HDPE) plastic bag, (25cm x 50cm). All PBRs were aerated from both top and bottom either with or without air stone for 10 days of *A. platensis* cultivation with daily monitoring of growth parameters. For bottom aeration, highest productivity was shown by Angular PBR with air stone (AS) at 0.090±0.002 $gL^{-1}d^{-1}$; while for top aeration, the productivity was shown highest by Cylindrical PBR aerated also with air stone (AS) at 0.071±0.001 $gL^{-1}d^{-1}$. Overall biomass of floating PBRs with and without air stone are significantly higher than land based PBRs. Regardless of having sophisticated designs for cultivation system, developing countries could use simple floating PBRs for commercial applications.

Keywords: *Spirulina*, *Arthrospira platensis*, floating, photobioreactor, PBR, mixing, bubble aerations.

Introduction

To date, studies on developing advanced technologies in microalgae cultivation systems have been conducive especially for enclosed photobioreactors. Most photobioreactor designs are land based, sophistically complicated, high initial cost, expensive preservation, exposed to high contamination risk and normally reserved to culture high value microalgae (Ugwu et al. 2008). The advantages of microalgae production in photobioreactor are noticeable (Grima et al. 1999) compared to other systems but cost, complicated designs and difficulties in maintenance making it at disadvantage producing pure microalgae (Tredici & Materassi 1992; Camacho Rubio et al. 1999; Ogbonna et al. 1999; Ugwu et al. 2002). To a certain extent of open systems, culture purity might decline distress after few generations of cultivation cycle (Borowitzka 1999). Less study were conducted on enclosed photobioreactor system for outdoor condition in Malaysia and mostly were developed on small scales. Most pilot projects for commercial microalgae cultivation operated using different types of photobioreactor system. In 2006, Algae International Sdn. Bhd., a pioneer company leading in microalgae cultivation in Malaysia has published a closed low-cost system of growing algae efficiently using a cultivation system known as Floating Bed Method (FBM) photobioreactors and has been patented few years after experimented and pilot studies (The Star Online 2011). Since 2006, this company has grown and has still producing Spirulina in totally enclosed floating photobioreactors. The FBM photobioreactor were fabricated with flexible custom made plastic materials which are expensive and puncturable. By all mean, simple floating photobioreactors design are without paddle wheel, pumps or impeller to circulate growth medium, no cooling mechanism and no mechanism to expel oxygen build ups from excess growth medium. There are insufficient data published on growth conditions of microalgae in comparison of floating photobioreactor design (Samson & Leduy 1985; Hu et al. 1996; Ugwu et al. 2008). A floating flexible photobioreactor project called offshore membrane enclosures for growing algae (OMEGA) system was deployed for growing microalgae efficiently using wastewater in the ocean environment (Wiley et al. 2013). Also, this project was carried out to investigate the technical feasibility of floating cultivation algae system towards commercial applications.

In 2012, another company in Malaysia Algaetech International Sdn. Bhd. has committed on using the concept of bio remediation system for sewage treatments with tubular photobioreactor system (Algaetech 2012). Private Limited Raman University has advanced the treatment of swine wastewater with microalgae cultivation technology system (Guang Ming Daily 2012). A closed lab-scaled photobioreactor has been newly developed and studied as Tenaga Nasional Berhad (TNB) researcher’s forthcoming ideas on developing sequestration of carbon system (Supramaniam et al. 2012). Recently in Orlando, Florida, Proterro Inc. has received patent on unique photobioreactor made off shelf plastic components as they were developing technology producing transgenetic cyanobacteria from fermentation-ready sucrose. The system was described having integrated systems for optimizing light, water supply and sugar collection (Ethanol Producer 2014). Usually photobioreactors were designed in small scale as preliminary models to be accessed on practicality of the system. In facts, there are higher odds of difficulties while going through the scalability of the new invented system for microalgae cultivation (Miron et al. 1999). As mentioned above, progress on research and developments were carried out on enclosed photobioreactors since decades ago. The evolutions started and continued growing into different kinds of tubular photobioreactors (Torzillo et al. 1993; Lee 2001; Molina et al. 2001; Morais et al. 2007), flat-plate photobioreactors (Milner 1953; Hu et al. 1996), and vertical-column photobioreactors (Choi et al. 2003; Garcı´a-Malea Lopez et al. 2006; Kaewpintong et al. 2007). Performance of the photobioreactors change on scale up (Camacho-Rubio et al. 1999), except most variable like light-dark cycling time of fluid interchange, mass transfer of oxygen and carbon dioxide, illumination level of the vessel were to held constantly upon scaling up (Grima et al. 1999).

Aeration supply and mixing efficiency are other fundamental variables were considered affecting growth of microalgae inside the photobioreactor system. Mixing methods are vital in order to assure fertilizer medium were to be distributed evenly in the culture inside enclosed photobioreactor. For open channel type of cultivation like open pond, paddle wheels were used for mixing as it can be located in big area of cultivation (Borowitzka 1999). With proper efficient mixing, the growth of Spirulina can be improved as more microalgae cells exposed to sunlight for photosynthesis process. Typical airlift bubble type reactors (vertically) usually have bigger bubble size used to supply aeration inside culture (Fu et al. 2004). However in this study, simple bubble mixing were supplied either using air stone producing mini bubbles or without air stone. Several tests were conducted in order to reaffirm the optimal supplementation of aeration or mixing level for microalgae in photobioreactor. Different sizes of bubbles are expected to give out different outcomes in the productivity of Spirulina. Another major function of mixing or aeration is to maintain the balance of gas exchange inside photobioreactors (Wang et al. 2012). Excessive or extreme agitation or aeration could affect the growth of microalgae cell particularly for Spirulina. Bubbles from the aeration could be breaking the long chained filament of Spirulina cells into short pieces while promotes other microalgae species to grow inside the photobioreactors (Suh & Lee 2003; Vunjak-Novakovic et al. 2005).

Mixing for other specific designs will need few other mechanical supports like pumping machine or static mixers to accomplish best outcomes for the productivity and gas exchange efficiency. Optimal mixing rate with higher light intensity has increased the output rates; however much less or too high mixing rate resulted in lower productivity or damages to cells and could reduced the output rate respectively as reported for flat plate photobioreactor cultivation (Hu & Richmond, 1996). The condition of high humidity level in Malaysia is leaving no choices but to use fully closed photobioreactor to cultivate microalgae instead of using open channel. Mixing power, oxygen buildups and capacity of CO_2 gaseous storage have significant effects towards the species productivity as it varies till meet required favorable conditions so to be maintained (Weissman et al., 1988). All in all, different photobioreactor designs were employed with the aim to examine the effects of simple mixing variations of bubble aerations on productivity and growth rate of *A. platensis.* These workings are focused on improving mixings efficiency of new simple water based cultivation system, cost effective made materials of enclosed photobioreactor to be generally compared to the performance of simple land based photobioreactor.

Materials and Methods

Preparation of Reference Growth Medium

Kosaric medium was prepared (Tompkins et al. 1995) with minor modification using commercial fertilizer (g L^{-1}): 5.0 $NaHCO_3$, 0.25 NaCl, 0.1 $CaCl_2$, 0.2 $MgSO_4.7H_2O$, 0.221 Urea, 0.07 H_3PO_4, 0.242 KOH, 0.02 FeSO4.7H2O and 0.5 mL/L of trace metals solution composed of following elements (g L^{-1}): 2.86 H_3BO_4, 1.81 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, 0.08 $CuSO_4.5H_2O$, 0.01 MoO_3, and 0.01 $COCl_2.6H_2O$ (Sukumaran et al. 2014). Urea was added to culture medium by using fed-batch method (pulse fertilization) (Danesi et al. 2002).

Preparation of land based photobioreactor designs

A vertically cylindrical land based photobioreactor design (working volume of 20 L) were constructed and located at the sheltered courtyards of Biology Department. Simple land based vertically cylindrical photobioreactor design (**Fig. 1**) were prepared at dimension: : π (20.5cm)^2 (50cm) using materials: iron wire strings, wire net 50cm x 50cm, 25cm x 50cm transparent High-density polyethylene (HDPE) plastic bag, air tubes and air stone. The wire net was circled and tied together with wire strings at both end vertically as a support structure for the plastic bag. Top part of the photobioreactor was covered with a hard plastic sheet, 30cm x 30cm and clipped to the wire net. Plastic bag were then placed inside the standing wire net carefully without any holes and scratches.

Fig. 1: Prepared land photobioreactors (PBR).

Preparation of floating water-based photobioreactor (PBR) designs

Photobioreactors (working volume of 20L) were designed from cheap, long-lasting, tough and transparent materials for high light illumination. There were two designated shapes of floating water based photobioreactors (PBR); angular and cylindrical floating photobioreactors. Both designs were developed with combination of several pieces (5L) plastic bottles (PET) with dimensions: Angular PBR, 16cm x16cm x110cm; Cylindrical PBR, $\pi r^2 h = \pi\ (10\text{cm})^2\ (78\text{cm})$ (**Fig.2**). The aeration was designated to be different for each shape of the photobioreactor either supplied from top or from bottom. The photobioreactors were also designed to have sufficient area for gaseous flow exchange in and out photobioreactors without having the design totally open to the environment. Only one closed port/outlet was designed available purposely for nutrient feeds, collecting samples and harvesting. Invariable size and material of buoyant were attached to all photobioreactors for better floatation on water surface. Prepared photobioreactors were located in outdoor environment condition under direct sunlight penetration.

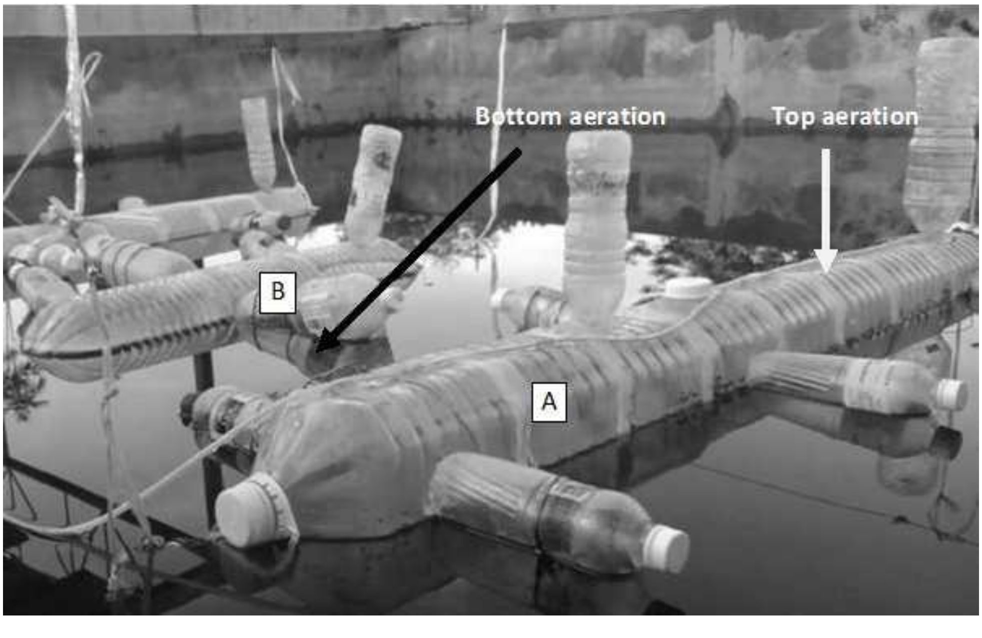

Fig. 2 Prepared floating photobioreactors (PBR) (A: Angular, B: Cylinrical).

Culture parameter and Growth Measurement

Light intensity in (µmol.m^{-}2.s^{-1}): (Licor Li-250), culture temperature (°C) (Fisher Scientific) and pH of the culture medium: (Mettler Toledo Model 330) were recorded daily. Growth performance parameter determination based on the following methods: Optical density (absorbance: at spectral range of 620nm) (Chu et al. 1995): (Hitachi U-1900). Dry weight following method in Borowitzka (1991), was analyzed daily. Productivity and specific growth rate calculated following formulas as mentioned below. Data analysis using SPSS software (Version 21) through One-way ANOVA, Tukey HSD and bivariate correlation for comparison between growth parameters readings.

Productivity

Productivity was calculated using the following equation according to Danesi et al. (2011):

$P_x = (X_m – X_i)(T_c)^{-1}$

where: P_x = productivity (g L^{-1} day^{-1})

X_i = initial biomass concentration (g L^{-1})

X_m = maximum biomass concentration (g L^{-1})

T_c = cultivation time related to the maximum biomass concentration (days)

Specific growth rate

Specific growth rate (µ) was calculated by the following formula according to Markou et al (2012):

$\mu = (\ln X_m - \ln X_i)(T_c)^{-1}$

where: X_i = initial biomass concentration (g L^{-1})

X_m= maximum biomass concentration (g L^{-1})

T_c = cultivation time related to the maximum biomass concentration (days)

Specific culture protocols for mixing variations *(with and without air stone; top; bottom)*

The experiment in Malaysia was conducted mainly at the Plant Physiology Lab and sheltered courtyard, Biology Department, Faculty of Science, Universiti Putra Malaysia. The blue-green alga *Arthrospira platensis* obtained from the algae culture collection at the Plant Physiology Lab, University Putra Malaysia. Stock cultures of the *A. platensis* were adapted to 3 different photobioreactors designs (Angular, Cylindrical and Land PBR) and were maintained as separate stock cultures with modified Kosaric medium by using urea as mentioned above. After 3 cycles of acclimatization phase, prepared *A. platensis* achieved sufficient adaptation for stable growth during experiment. 10% of *A. platensis* seeds (2L) from stock culture were cultivated in all triplicates sets of the photobioreactors including mixture of prepared media and filtered tap water (18L). All photobioreactors was aerated with standard diaphgram aquarium air pump (SONIC P-125, 85L/min, 0.04MPa) with standardized aeration rate at 0.7 L L^{-1} min^{-1} and placed in outdoor conditions for direct light sources. Aeration was supplied with air stone (AS) for small size of bubbles and without air stone (WAS) for big bubbles. The point of introduction of bubbles sources inside photobioreactors were placed either on top (above bottom base) or at the bottom of the photobioreactors. Inside Land, Angular and Cylindrical PBR, aeration supplied and mixing flows were as sketched for top and bottom aeration (refer **Fig. 3**: a, b, c & d). Two aeration points were placed at the front and at the back for each floating photobioreactor, seeing as it was designed horizontally unlike land based photobioreactor design with only one aeration point.

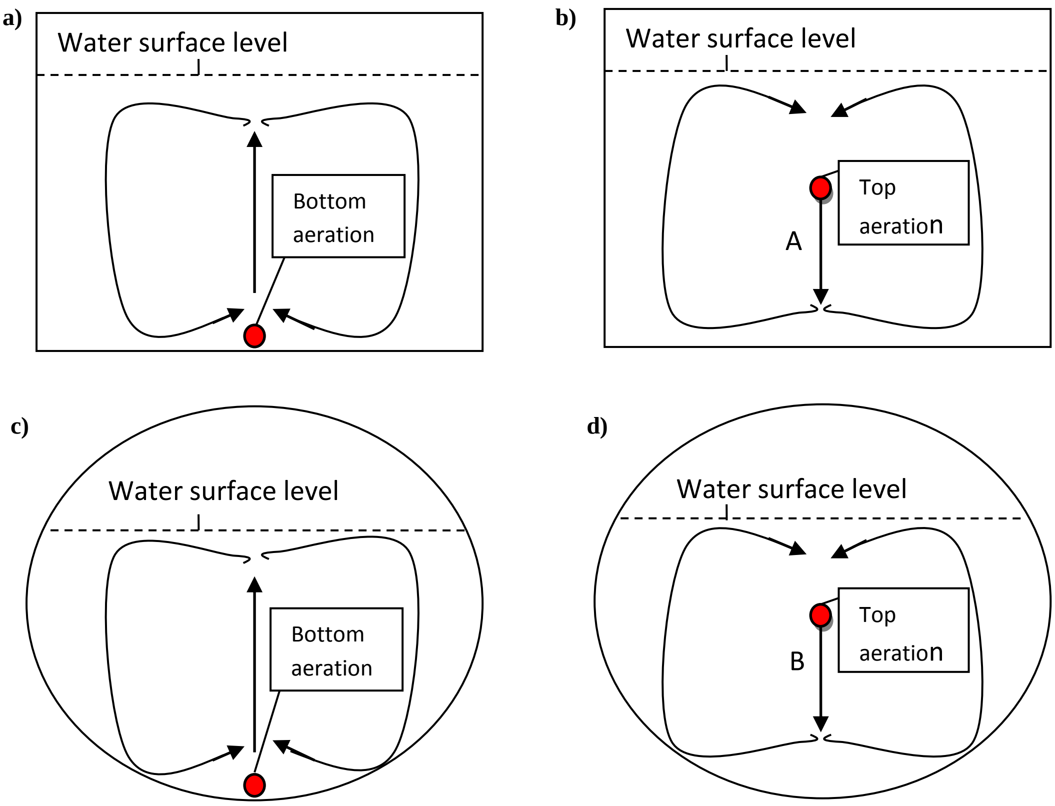

Fig. 3 Schematic diagram of aeration mixing flows positioned at a) bottom aeration in Land & Angular PBR; b) top aeration in Land and Angular PBR (A: 6cm±0.5cm); c) bottom aeration in Cylindrical PBR; d) top aeration in Cylindrical PBR (B: 4cm±0.5cm).

Results and Discussion

Growth of *A. platensis* in simple photobioreactors (PBR) with mixing variation

Optical density

(i) *Bubble aerations from bottom* (*With and without air stone*)

Mixing effects for bubble aerated from bottom inside photobioreactors (PBR) with air stone (AS) and without air stone (WAS) on the growth of *A. platensis* in terms of optical density were presented in **Fig. 4**. Initial average readings ABS (optical density) for cultures with AS and WAS were at 1.031±0.052, 0.799±0.005 (Angular PBR); 0.878±0.106, 0.663±0.004 (Cylindrical PBR) and 0.590±0.039, 0.523±0.032 (Land PBR as control) respectively (refer Figure 4). Highest absorbance achieved for mini-bubble and big bubble (values are presented as Mean ± SE) on Day 10 were 2.897±0.022, 2.106±0.014 for Angular PBR; 2.201±0.112, 2.389±0.03 for Cylindrical PBR and 1.443±0.032, 0.905±0.123 for Land PBR in that order. Angular PBR with AS had significantly higher density ($p < 0.05$) compared to other two groups: (AS and WAS of Cylindrical PBRs and Angular PBR for WAS; Land PBRs for AS and WAS). Both floating Cylindrical PBRs aerated with AS and WAS and Angular PBR of WAS had significantly higher density ($p < 0.05$) than both AS and big WAS of Land PBRs. For total 10 days of cultivation period, the highest average mean ± SE of the optical density was achieved with culture treated in Angular PBR (AS), 1.977±0.123.

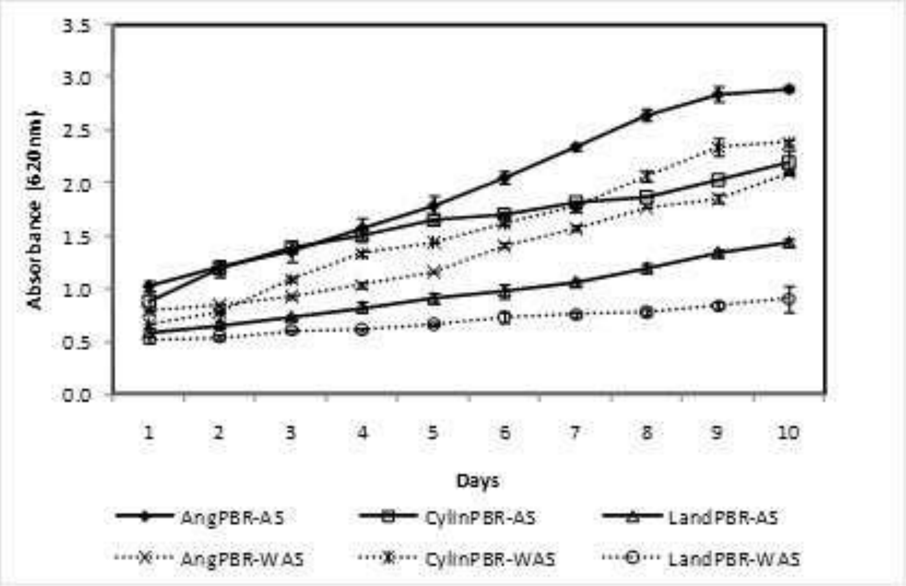

Fig. 4 Absorbance of *A. platensis* grown with bottom bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular; Cynd-Cylindrical; Land Photobioreactors-control) for 10 days. Values are presented as Mean ± SE (n = 3).

(ii) *Bubble aerations from top* (*With and without air stone*)

The initial mean ± SE density (absorbance, ABS) recorded for on the first day for culture treated with air stone (AS) and without air stone (WAS) were at 0.413±0.005, 0.482±0.010 (Angular PBR); 0.865±0.034, 0.363±0.004 (Cylindrical PBR); 0.260±0.010, 0.428±0.017 (Land PBR) (ABS) respectively (refer **Fig. 5**). On Day 10, culture treated with AS in Cylindrical PBR gave the highest mean ± SE (620 nm) density at 2.097±0.045 and the lowest mean ± SE of density was observed on Land PBR with AS, 0.716±0.011. There are three distinct growth patterns for mixing/aeration using AS and WAS aerated from top inside photobioreactors. AS aerated Cylindrical PBR had significantly higher density ($p < 0.05$) than another two groups (both Angular PBR of AS and WAS, Cylindrical PBR with WAS, and Land PBR with WAS) and (both Land PBR with AS and WAS). There are no significant differences ($p > 0.05$) within these two groups respectively.

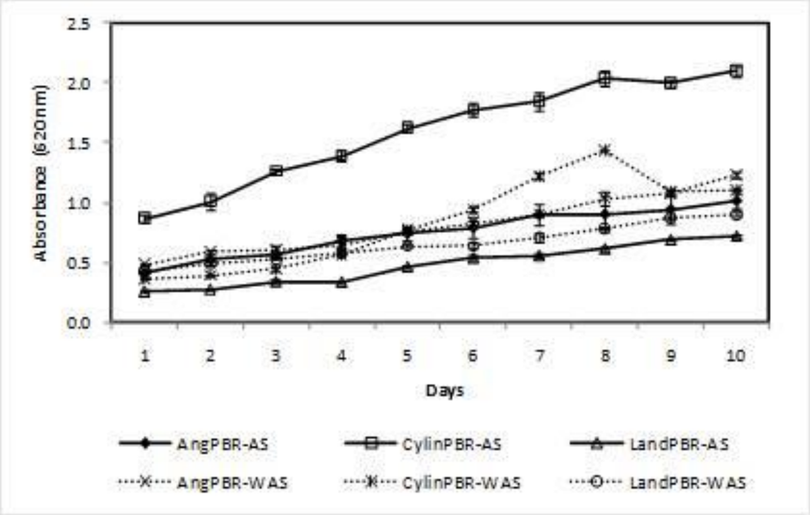

Fig. 5 Absorbance of *A. platensis* grown with top bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular; Cylin-Cylindrical; Land Photobioreactors-control) for 10 days. Values are presented as Mean ± SE (n = 3).

Biomass Dry weight

(i) *Bubble aerations from bottom* (*With and without air stone*)

For the dry weight (g L^{-1}), highest mean ± SE achieved on Day 10, was obtained with air stone (AS) aerated culture in

Angular PBR, 1.441±0.041 and the lowest was Land PBR without air stone (WAS) aeration, 0.444±0.016 (**Fig. 6**). The highest average mean ± SE of dry weight (g L^{-1}) was observed on Angular PBR with AS aeration, 0.957±0.059 is not significantly different ($p > 0.05$) to AS aerated Cylindrical PBR. However, dry weight (g L^{-1}) collected from AS aerated Angular PBR is significantly higher ($p < 0.05$) compared to other aerated photobioreactors; Angular PBR-WAS, Cylindrical PBR-WAS and Land PBR-AS and WAS. Overall results shown that biomass dry weight (g L^{-1}) for Land PBR aerated with AS and WAS from bottom are significantly lower ($p < 0.05$) than floating photobioreactors with AS and WAS (Angular and Cylindrical PBR).

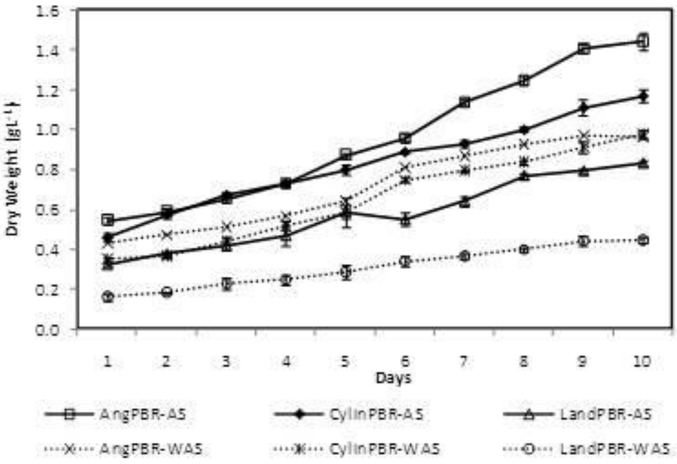

Fig. 6 Dry weight (g L^{-1}) of *A. platensis* grown with bottom bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular; Cynd-Cylindrical; Land Photobioreactors-control) for 10 days. Values are presented as Mean ± SE (n = 3).

(ii) *Bubble aerations from top* (*With and without air stone*)

Initial mean ± SE of dry weight (g L^{-1}): 0.254±0.008, 0.609±0.008; 0.461±0.005, 0.227±0.018; 0.150±0.022 and 0.231±0.019 g L^{-1} respectively for all triplicate sets of air stone (AS) and without air stone (WAS) aeration from top (Angular, Cylindrical & Land PBR) (**Fig. 7**). On Day 10, the highest mean ± SE of biomass dry weight were obtained with AS aerated Cylindrical PBR, 1.167±0.017. Second highest recorded on dry weight (g L^{-1}) was from *A. platensis* culture aerated with WAS in Angular PBR, 0.829±0.041. The lowest observed dry weight (g L^{-1}) shown by *A. platensis* culture aerated with AS in Land PBR, 0.264±0.035. Average mean ± SE of dry weight (g L^{-1}) were achieved highest with AS aerated Cylindrical PBR, 0.816±0.047 was significantly higher ($p < 0.05$) than other AS and WAS aerated photobioreactors. For top bubble aerations, results shown that the biomass dry weight (g L^{-1}) for both AS and WAS aerated Land PBR are significantly lower ($p < 0.05$) than floating photobioreactors with AS and WAS (Angular and Cylindrical PBR).

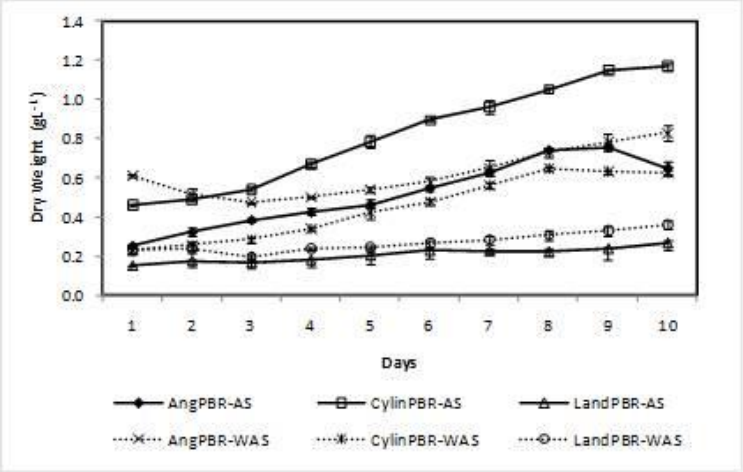

Fig. 7 Dry weight (g L^{-1}) of *A. platensis* grown with top bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular; Cynd-Cylindrical; Land Photobioreactors-control) for 10 days. Values are presented as Mean ± SE (n = 3).

Based on **Table 1**, growth parameters between absorbance (ABS) and biomass dry weight between bottom and top bubble aeration are significantly ($p < 0.01$) correlated. Also, it can be concluded that each groups of (biomass dry weight vs absorbance), (absorbance of Bottom and Top Aeration) and (biomass dry weight of Bottom vs Top Aeration) are significantly correlated ($p < 0.01$) within groups respectively.

Table 1 Results of correlation between growth parameters for bottom and top bubble aerations (AS-air stone & WAS-without air stone) in different simple photobioreactors (Ang: Angular, Cylin: Cylindrical & Land).

PBR-Bubble Type	Biomass & ABS of Bottom Aeration (pearson correlation, r)	Biomass & ABS of Top Aeration (pearson correlation, r)	ABS of Bottom & Top Aeration (pearson correlation, r)	Biomass of Bottom & Top Aeration (pearson correlation, r)
Ang-AS	0.983*	0.770*	0.807*	0.943*
Cylin-AS	0.978*	0.955*	0.960*	0.971*
Land-AS	0.963*	0.491*	0.937*	0.527*
Ang-WAS	0.965*	0.800*	0.972*	0.802*
Cylin-WAS	0.956*	0.955*	0.879*	0.957*
Land-WAS	0.941*	0.781*	0.963*	0.763*

* Correlation is significant at the 0.01 level (2-tailed).

Table 2 Results of productivity (g L^{-1} d^{-1}) and specific growth rate (μ d^{-1}) of *A. platensis* grown with bottom and top bubble aerations (AS-air stone & WAS-without air stone) in different simple photobioreactors (Ang: Angular, Cylin: Cylindrical & Land).

PBR-Bubble Type	Productivity with Bottom Aeration (g L^{-1} d^{-1})	Specific growth rate with Bottom Aeration (μ d^{-1})	Productivity with Top Aeration (g L^{-1} d^{-1})	Specific growth rate with Top Aeration (μ d^{-1})
Ang-AS	0.090 ± 0.002^{a}	0.098 ± 0.089^{a}	0.040 ± 0.002^{a}	0.094 ± 0.135^{ab}
Cylin-AS	0.071 ± 0.001^{b}	0.094 ± 0.050^{a}	0.071 ± 0.001^{b}	0.093 ± 0.119^{ab}
Land-AS	0.051 ± 0.000^{c}	0.095 ± 0.066^{a}	0.011 ± 0.001^{c}	0.056 ± 0.045^{abc}
Ang-WAS	0.054 ± 0.001^{c}	0.081 ± 0.094^{a}	0.022 ± 0.003^{c}	0.031 ± 0.160^{bc}
Cylin-WAS	0.063 ± 0.001^{bc}	0.103 ± 0.103^{a}	0.040 ± 0.000^{a}	0.101 ± 0.019^{a}
Land-WAS	0.028 ± 0.000^{d}	0.089 ± 0.009^{a}	0.013 ± 0.000^{c}	0.044 ± 0.019^{bc}

* Each value is presented as Mean ± SE (n = 3). Means within each column with different letter (a-d) differs significantly ($p < 0.05$).

Table 3 Results of correlation between growth parameters (absorbance & biomass dry weight of bottom and top bubble aerations (AS-air stone & WAS-without air stone) vs light intensity).

PBR-Bubble Type	ABS Bottom vs Light (pearson correlation, r)	ABS Top vs Light (pearson correlation, r)	Biomass Bottom vs Light (pearson correlation, r)	Biomass Top vs Light (pearson correlation, r)
Ang-AS	0.656*	0.721*	0.698*	0.599*
Cylin-AS	0.667*	0.604*	0.653*	0.672*
Land-AS	0.660*	0.657*	0.740*	0.323*
Ang-WAS	0.671*	0.685*	0.586*	0.539*
Cylin-WAS	0.647*	0.582*	0.651*	0.673*
Land-WAS	0.658*	0.740*	0.702*	0.685*

* Correlation is significant at the 0.01 level (2-tailed).

pH values observed for bottom and top bubble aeration (AS and WAS) in the photobioreactors (Angular, Cylindrical & Land) were presented in mean ± SE (n = 3) in **Fig. 8** and **Fig. 9** respectively. For bottom bubble aerations, average pH were initially recorded for air stone (AS) and without air stone (WAS) aerated culture in (Angular PBR: 9.64±0.012, 9.60±0.044); (Cylindrical PBR: 9.33±0.021, 9.69±0.088) & (Land PBR: 9.52±0.024, 9.34±0.006). Meanwhile the initial average pH for top bubble aerations (AS and WAS) of culture inside all photobioreactors were in range of 9.0 to 10.0 and has achieved (Angular PBR: 10.23±0.032, 10.28±0.058); (Cylindrical PBR: 10.34±0.075, 10.28±0.028) & (Land PBR: 9.76±0.085, 9.96±0.019) on the last day of cultivation, Day 10. The changes of pH were closely related to the photosynthetic activities.

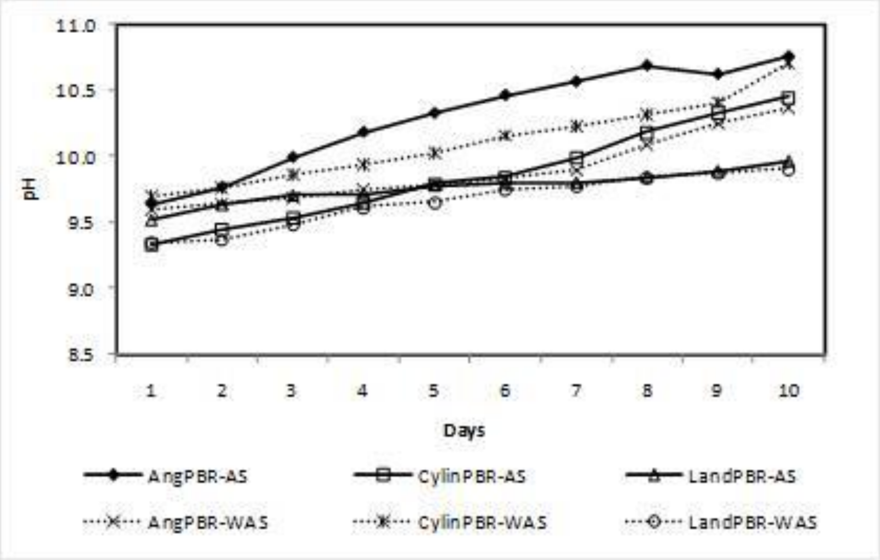

Fig. 8 pH of grown *A. platensis* with bottom bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular, Cynd-Cylindrical & Land as control) for 10 days. Values are presented as Mean ± SE (n = 3).

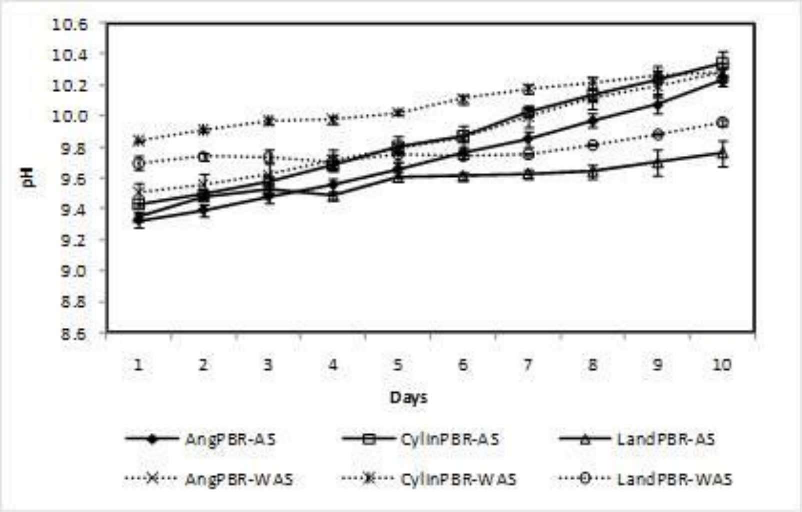

Fig. 9 pH of grown *A. platensis* with top bubble aeration supplied using (AS-air stone & WAS-without air stone) in different simple photobioreactors (PBR) (Ang-Angular, Cynd-Cylindrical & Land as control) for 10 days. Values are presented as Mean ± SE (n = 3).

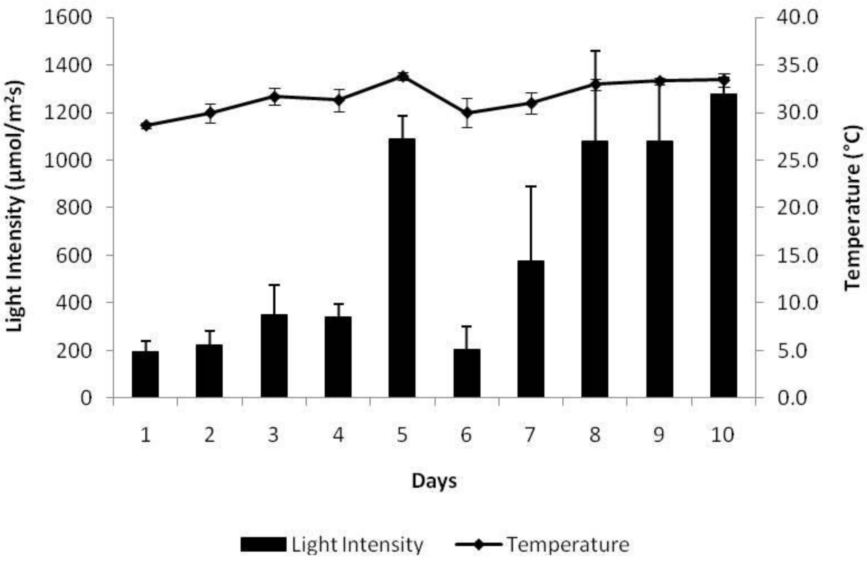

Fig. 10 Average light intensity (µmol/m^{2}s) and temperature (°C) for the 10 days of *A. platensis* cultivation with bottom and top aeration of (mini-bubble & big bubble) in different simple photobioreactors (PBR). Values are presented as Mean ± SE (n = 3).

Mixing system varies depends on the microalgae cultivation system. As for open channel system, paddle wheels were used for cultivation mixing (Vonshak 1997b). With different bubble aeration mixing supplied through air stone and without air stone, floating photobioreactors have resulted significantly higher yield ($p < 0.05$) compared to land photobioreactors under same environmental factors. Productivity difference and the specific growth rate between photobioreactors were presented on **Table 2**. Clearly, aeration supplied from bottom with air stone producing mini bubbles indicated highest productivity for floating photobioreactors.

Theoretically, bubble aerations that were supplied from bottom are to lift the *A. platensis* microalgae cells upwards to circulate culture inside photobioreactors. Whereas, air bubbles that were blown or projected from the top were expected to force cells downwards and drive all the cells to the sides by passing it together to the top again with the bubbles movements. Aeration from bottom has signified higher productivity, 0.090 ± 0.002 of Angular PBR aerated with air stone and has also achieved high specific growth rate, 0.103 ± 0.103 with Cylindrical PBR aerated without air stone. The top aeration force probably was not adequate to fully circulate culture cells inside photobioreactors. Aeration supplied from top had bubbles emerge from point of introduction at 4cm above bottom surface caused deficient aeration to zone below as bubbles quickly rise upwards to the surface. Therefore, zone below the point of introduction gets limited circulation. Most bubbles drawn out from top were unable to reach bottom zone, thus microalgae cells at these bottom region were left undisturbed continued receiving limited circulation and exposure of sunlight. These could be the reasons of low productivity of top aerated photobioreactors. A lesser amount of time period of exposure towards sunlight has also decreased the *A. platensis* culture photosynthetic activities significantly (Hu et al. 1996; Hu et al. 1998; Oncel & Vardar 2008). With less circulations, nutrient uptakes were less distributed accurately, which later affected the productivity rate of *A. platensis* (Yuan et al. 2011).

The culture depth inside photobioreactors was a lot shallower than airlift bioreactor or bubble column reactor. Optimal aeration rate was adjusted for *A. platensis* culture, to avoid cells stress. Bigger size and strong bubbles could cause breakage of filaments and rapid circulations could also cause limited time of light exposure evidently by the data obtained and from previous studies (Chisti, 1989; Oncel & Vardar, 2008). Big bubbles created violent movements while, small bubbles created gentle movements and promote better exchange of gaseous. Extreme aeration flows would be harmful as claimed from several indications of short trichomes appearances and yellowish foam developments during cultivation periods. Also, minimal aeration rate suggested for air bubbling was at the rate of 0.06 L L^{-1}min^{-1} to keep algal entirely circulated. Peak oxygen productions related to photosynthetic activities were shown at slightly above 4 L L^{-1}min^{-1} were recommended as optimal mixing rate for flat-plate photobioreactor (Hu & Richmond, 1996). However in this experiment due to different photobioreactor shapes and designs, there were no occurrences of yellowish foams during cultivation periods.

In this study, mixing system with bubble aeration is uncomplicated, require less operating energy, cost effective and can be easily setup to either water or land based simple designed photobioreactors. Some photobioreactor systems may need appropriate complex advanced technology to proficiently embrace the cultivation system for longer periods, such as paddle wheel (Hase et al. 2000), static mixer (Ugwu et al. 2003), impeller (Pruvost et al. 2006), tank stirrer (Singh & Sharma 2012) and swirl vanes (Wiley et al. 2013). Throughout this experiment, simple mixing has indicated constructive results to the growth rate and productivity of *A. platensis*. Also, there was no negative implication on implementing the simple mixing system in closed photobioreactors.

Mixing are also vitals in order to decrease boundary layers around the cells, balancing gaseous exchange inside cultured photobioreactor and nutrient uptakes for increasing growth rates (Grobbelaar 1991). Dissolved oxygen gas accumulations inside closed photobioreactors were seen at disadvantages depends on the bioreactor designs. Thus for solution, additional methods in terms of mixing forces or degassing sections were invented particularly based on the bioreactor designs to improve the gaseous exchange limitations (Torzillo et al. 1997; Converti et al. 2006). Learning from previous studies, the floating photobioreactor were designed to have cell circulations with bubble aerations to avoid excess oxygen gas build ups. Besides, the floating photobiorectors were also occupied simply with air release compartment on top of the photobioreactor to assure sufficient areas for gaseous exchange.

The aeration point from top was introduced at different depth above bottom basement inside all photobioreactors (PBR). Only differences in shapes of photobioreactors that is left could possibly be another main cause of varies culture circulation effectiveness. From the results gained, Cylindrical PBR with air stone enhanced better circulation flows following its shape and gave higher productivity ($p < 0.05$) compared to other PBRs. Volume, size and design of photobioreactors affects water circulation which later involves the circulation operation cost and its design of mixing system (Borowitzka 2005). Vonshak (1997b) also reported dissimilarity in mixing flows and yield between small pond and large pond microalgae cultivation. An increase to the aeration rate above 1.5 cms^{-1} has resulted negatively to the growth of diatom in 3L culture volume of both airlift and bubble column reactor (Monkonsit et al. 2011). Disproportionate between circulations flow rate and culture volume size and bioreactor designs could cause disturbance to the growth of microalgae. In small size bioreactor, extreme bubbling aeration rate could disrupt photosynthetic activities as light were less penetrated hindered by bubbles foam layer (Gavrilescu & Tudose 1998). To some extent, mixing systems used in experimental designs are scarcely used for commercial application as many did not comply in terms of scalability and practicality. With simple and optimal mixing force, good quality of Spirulina can highly achieved through efficient methods and apparatus.

Acknowledgement

The authors thank the University Agriculture Park and Plant Physiology Lab, Biology Department, University Putra Malaysia for their technical assistance and support.

References

Algaetech International Sdn. Bhd., September 21 2012. http://algaetech.com.my/v1/category/press-release/

Borowitzka, M.A. 1991 The algae: Their biology and limits to growth 71-94 pp. Proceedings, Seminar Held at Murdoch University, Western Australia, November 29

Borowitzka, M.A. 1999 Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotechno.* **70** (1– 3): 313–321

Borowitzka, M.A. 2005 Culturing microalgae in outdoor ponds. In: R.A. Andersen (Ed.), Algal culturing techniques, pp. 205–218 Elsevier academic press, London.

Camacho Rubio, F., Acien Fernandez, F.G., Sanchez Perez, J.A., Garcia Camacho, F., Molina Grima, E. 1999 Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. *Biotechnol. Bioeng.* **62** (1): 71–86

Chisti, Y., Grima, E.M., Belarbia, E.H., Fernandez, F.G.A. & Medina, A.R. 2003 Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances* **20**: 491–515

Choi, S.L., Suh, I.S. & Lee, C.G. 2003 Lumostatic operation of bubble column photobioreactors for *Haematococcus pluvialis* cultures using a specific light uptake rate as a control parameter. *Enzyme Microb.Technol.* **33**: 403–409

Chu, W.Y., Phang, S.M., and Goh, S.H. 1995 Influence of carbon source on growth biochemical composition and pigmentation of *Ankistrodesmus convolutus*. *J. of Appl. Phy*. **7**: 59-64

Converti, A., Lodi, A., Del Borghi, A., Solisio, C. 2006 Cultivation of Spirulina platensis in a combined airlift-tubular reactor system. *Biochemical Eng J* **32**: 13–18

Danesi, E.D.G., Rangel-Yagui, C.O., Carvalho, J.C.M. & Sato, S. 2002 An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. *Biomass Bioenerg.* **23**: 261–269

Danesi, E.D.G., Rangel-Yagui, C.O., Sato, S., & Carvalho, J.C.M.D. 2011 Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. *Brazilian Journal of Microbiology* **42** (1): 362-373

Ethanol Producer Magazine, 2014 ethanolproducer.com/articles/10766/proterro-receives-patent-on-unique-photobioreactor

Fu, C.C., Lu, S.Y., Hsu, Y.J., Chen, G.C., Lin, Y.R. & Wu, W.T. 2004 Superior mixing performance for airlift reactor with a net draft tube. *Chemical Engineering Science* **59**: 3021-3028

Garcı´a-Malea Lo´pez, M.C., Del R´ıo Sa´nchez, E., Casas Lo´pez, J.L., Acie´n Ferna´ndez, F.G., Ferna´ndez Sevilla, J.M., Rivas, J., Guerrero, M.G. & Molina Grima, E. 2006 Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors. *J.Biotechnol.* **123**: 329–342

Gavrilescu, M. & Tudose, R.Z. 1998 Modelling of liquid circulation velocity in concentric-tube airlift reactors. *Chemical Engineering J.* **69**: 85-91

Grima, E. M., Acie´n Ferna´ndez, F. G. A., Garcia Camacho, F. G. & Chisti, Y. 1999 Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.* **70** (1– 3): 231–247

Grobbelaar, J.U. 1991 The influence of light/dark cycles in mixed algal cultures on their productivity. *Bioresource Technol.* **38**: 189-194

Guang Ming Daily, August 9 2012. http://www.guangming.com.my/node/142893?tid=23

Hase, R., Oikawa, H., Sasao, C., Morita, M., Watanabe, Y. 2000 Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai City. *J. Biosci. Bioeng.* **89**: 157–163

Hu, Q., & Richmond, A. 1996 Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *Journal of Applied Phycology* **8**: 139-145

Hu, Q., Guterman, H. & Richmond, A. 1996 A flat inclined modular photobioreactor for outdoor mass cultivation of phototrophs. *Biotechnol. Bioeng.* **51**: 51–60

Hu, Q. Zarmi, Y. & Richmond, A. 1998 Combined effects of light intensity, light-path and culture density on output rate of *Spirulina platensis* (Cyanobacteria). *Eur J Phycol* **33**: 165–171

Kaewpintong, K., Shotipruk, A., Powtongsook, S. & Pavasant, P. 2007 Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. Bioresource Technol. **9**: 288–295

Lee, Y.K. 2001 Microalgal mass culture systems and methods: Their limitation and potential. *Journal of Applied Phycology* **13**: 307-315

Markou, G., Chatzipavlidis, I., & Georgakakis, D. 2012 Effects of phosphorus concentration and light intensity on the biomass composition of *Arthrospira (Spirulina) platensis*. *World Journal of Microbiology and Biotechnology* **28** (8): 2661-2670

Milner, H.W. 1953 Rocking tray. In: J.S. Burlew (Ed.), Algal Culture from Laboratory to Pilot Plant, pp. 108 No. 600 Carnegie Institution, Washington, DC.

Miron, A.S., Gomez, A.C., Garcia Camacho, F.G., Grima, E.M. & Chisti, Y. 1999 Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *J. Biotechnol.* **70** (1-3): 249-270

Molina-Grima, E., Fernandez, J., Acien Ferna´ndez, F.G. & Chisti, Y. 2001 Tubular photobioreactor design for algal cultures. *Journal of Biotechnology* **92** (2): 113-131

Monkonsit, S., Powtongsook, S. & Pavasant, P. 2011 Comparison between airlift photobioreactor and bubble column for *Skeletonema costatum* cultivation. *Engineering Journal* **15** (4): 53-64

Morais, De M.G. & Costa, J.A.V. 2007 Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology* **129** (3): 439-445

Ogbonna, J.C., Soejima, T., Tanaka, H. 1999 An integrated solar and artificial light system for internal illumination of photobioreactors. *J. Biotechnol.* **70**: 289–297

Oncel, S. & Vardar Sukan, F. 2008 Comparison of two different pneumatically mixed column photobioreactors for the cultivation of *Arthrospira platensis (Spirulina platensis). Bioresources Technology* **99**: 4755-4760

Pruvost, J., Pottier, L., & Legrand, J. 2006 Numerical investigation of hydrodynamic and mixing conditions in a torus photobioreactor. *Chem. Eng. Sci* **61**: 4476–4489

Samson, R. & Leduy, A. 1985 Multistage continuous cultivation of bluegreen alga *Spirulina maxima* in the flat tank photobioreactors. *Can. J. Chem. Eng.* **63**: 105–112

Singh, R.N. & Sharma, S. 2012 Development of suitable photobioreactor for algae production—a review. *Renew Sustain Energy Rev.* **16**: 2347–2353

Suh, I.S. & Lee, C.G. 2003 Photobioreactor engineering: design and performance. *Biotechnol. Bioprocess. Eng.* **8**: 313–21

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** (4): 235-250

Supramaniam, J., Palanisamy, K. & Nomanbhay, S.M. 2012 Study on the pH changes of microalgae (*Tetraselmis Chuii*) cultivated in newly developed closed photobioreactor using natural sunlight and artificial light. *Journal of Energy and Environment* **4** (1): 18-20

The Star Online, April 12 2011. http://www.thestar.com.my/story.aspx/?file=%2f2011%2f4%2f12%2flifefocus%2f8327270&sec=lifefocus

Tompkins, J., De Ville, M.M., Day, J.G. & Turner, M.F. 1995 Catalogue of Strains 14-73 pp. Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, Windermere Laboratory, Ambleside, Cumbria UK.

Torzillo, G., Carlozzi, P., Pushparaj, B., Montaini, E. & Materassi R. 1993 A two-plane tubular photobioreactor for outdoor culture of Spirulina. *Biotechnology and Bioengineering* **42** (7): 891-898

Torzillo, G. 1997 Tubular bioreactors. In: A. Vonshak (Ed.), Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology, pp. 101–115 Taylor and Francis, London.

Tredici, M.R. & Materassi, R. 1992 From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *J. Appl. Phycol.* **4**: 221-231

Ugwu, C.U., Ogbonna, J.C. & Tanaka, H. 2002 Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. *Appl. Microbiol. Biotechnol.* **58**: 600–607

Ugwu, C.U, Ogbonna, J.C. & Tanaka, H. 2003 Design of static mixers for inclined tubular photobioreactors. *J Appl Phycol.* **15**: 217–223

Ugwu, C.U., Aoyagi, H. & Uchiyama, H. 2008 Review-Photobioreactors for mass cultivation of algae. *Bioresources Technology* **99**: 4021-4028

Vonshak, A. 1997b Outdoor Mass Production of Spirulina: the basic concept. In: A. Vonshak (Ed.), Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology, pp. 79–99 Taylor and Francis, London.

Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I. & Merchuk, J.C. 2005 Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. *Ind.Eng.Chem.Res.* **44**: 6154–6163

Wang, B., Lan, C.Q. & Horsman, M. 2012 Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances* **30**: 904-912

Weissman, J.C., Goebel, R.P. & Benemann, J.R. 1988 Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnology and bioengineering* **31** (4): 336-344

Wiley, P., Harris, L., Reinsch, S., Tozzi, S., Embaye, T., Clark, K., McKuin, B., Kolber, Z., Adams, R., Kagawa, H., Richardson, T-M.J., Malinowski, J., Beal, C., Claxton, M.A., Geiger, E., Rask, J., Campbell, J.E. & Trent, J.D. 2013 Microalgae cultivation using offshore membrane enclosures for growing algae (OMEGA). *Journal of Sustainable Bioenergy Systems* **3**: 18-32

Yuan, X., Kumar, A., Sahu, A.K. & Ergas, S.J. 2011 Impact of ammonia concentration on *Spirulina platensis* growth in an airlift photobioreactor. *Bioresources Technology* **102** (3): 3234-3239