Optimization for cultivation of microalgae *Chlorella vulgaris* and lipid production in photobioreactor

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Abstract

Microalgae have been used as energy resources in recent decades to mitigate the global energy crisis. As the demand for pure microalgae strains for commercial use increases, designing an effective photobioreactor (PBR) for mass cultivation is important. *Chlorella vulgaris*, a local freshwater microalga, was used to study the algal biomass cultivation and lipid production using various PBR configurations (bubbling, air-lift, porous air-lift). The results show that a bubbling column design is a better choice for the cultivation of *Chlorella vulgaris* than an air-lift one. The highest biomass concentration in the bubbling PBR was 0.78 g/L while the air-lift PBR had a value of 0.09 g/L. Key operating parameters, including inner-tube length and bubbling flowrate, were then optimized based on biomass production and lipid yield. The highest lipid content was in the porous air-lift PBR and the air-lift PBR with shorter draft tube (35 cm) was also better than a longer one (50 cm) for algal cultivation, but the microalgae attached on the inner tube of PBR always occurred. The highest biomass concentration could be produced under the highest gas flowrate of 2.7 L/min whereas the lowest dry cell mass was under the lowest gas flowrate of 0.2 L/min. Besides, the biomass production in Day 10 in white LED was the highest (1.25 g/L) while blue LED, red LED and open pond were 0.80 g/L, 0.35 g/L and 0.58 g/L respectively.

Keywords: photobioreactor, *Chlorella vulgaris*, algal cultivation, lipid production, LED light

Introduction

The world is currently faced with energy challenges because of the instabilities in fossil fuel markets and the environmental impact of increasing exhaust emissions at a time when energy demand is exploding. These conditions accelerate the urgency of developing alternative renewable energy technologies. Biodiesel is a clean energy with fewer pollutants emitted to the atmosphere and with a high potential to solve the climate change caused by CO_2 emissions from fossil fuel combustion (IPCC, 2007; Zhao et al., 2011). The required biodiesel production is not only a promising renewable technology, but is also capable of lowering CO_2 emissions in the atmosphere to achieve environmental and economic sustainability (Chiu et al., 2009; Demirbas and Demirbas, 2011). Algae commercialization has numerous environmental, social and economic benefits. Microalgae can fix CO_2 with 10 to 50 times greater efficiency than terrestrial plants (Kumar et al., 2010; Wang et al., 2012). Algae have a much higher growth yield (10 to 100 times higher) compared with other biofuel sources such as corn (Greenwell et al., 2010). Some microalgae can also be used as a food additive (Spolaore et al., 2006). When combined with wastewater treatment process, sewage also provides a good nutrient source of nitrogen and phosphate for microalgae growth (Craggs et al., 2012; Kumar et al., 2010).

Two main reactor types (i.e. open-pond and closed PBR) are commonly used for microalgae cultivation. Open-pond systems are always located outdoors with natural light for illumination and limited control of

cultivation conditions, and operated at water depths of 15-30 cm. Closed PBRs are either naturally or artificially illuminated and aimed at mainly culturing single species of microalgae under controlled operating conditions. Closed PBRs are superior to open-pond systems in many aspects, such as lower water and CO_2 loss, less risk contamination of undesirable microorganisms (Jorquera et al., 2010; Posten, 2009; Wang et al., 2012; Yoo et al., 2011). However, they have not been fully scaled up due to the higher capital and operating costs compared to open-pond systems. The common closed PBR designs are flat plate, tubular, bubble column, and air-lift (Bitog et al., 2011; Posten, 2009; Wang et al., 2012). The maximal capture of solar energy is a major challenge in the design of PBR for commercial microalgal biomass production. Posten (2009) claimed that the PBR design is basically derived from the typical surface-to-volume ratio (SVR) of 80 m^2/m^3 to 100 m^2/m^3, and the larger the SVR, the higher the distribution of light to the PBR.

Light intensity is one of the essential limiting factors for cell growth in the photosynthesis process (Bitog et al., 2011; Janssen, 2002; Posten, 2009). For most bioreactors, the outer surface is the only place exposed to sunlight, called the photic zone. The rest that is unexposed to or with little sunlight penetration is called the dark zone. Different PBR geometries and designs result in varying hydrodynamic circulation and light utilization, which can affect light distribution and mixing inside the reactor. When microalgae are exposed to the light/dark zone within the PBR, the duration under the light/dark zone affects the biomass production of the PBR.

Apart from PBR geometry, light penetration in different areas of PBR also affects the growth rate of microalgae. Posten (2009) pointed out that light attenuation is caused by the absorption of light by the cells on the surface or by shading of the cells. In high-cell density cultures, mutual shading caused by the cells can occur. The surface microalgae absorb light energy, which results in lower light intensity inside a typical PBR (Park and Lee, 2001; Ranjbar et al., 2008). Wang et al. (2012) reported that a light limitation exists under low light intensity. When the intensity surpasses a critical level, light saturation and photo-inhibition may occur. They suggested several strategies to solve the light distribution problem in PBR, including improving mixing and limiting the length of the light path, such as in thin or small-diameter PBRs.

Microalgae grow effectively in PBRs under optimal biotic and abiotic conditions (i.e. pH, temperature, CO_2 exposure, lighting, and nutrients availability) (Acién Fernández et al., 2001; Barbosa et al., 2003; Sánchez Mirón et al., 2000; Yoo et al., 2012). Notably, nutrient availability in PBRs is significantly affected by aeration rate, gas hold-up and mixing of phototrophic cultivation. Dissolved oxygen (DO) also accumulates in algal cultures because of oxygen generation in photosynthesis. When excessive oxygen is present in a culture, the photosynthesis rate of algae is suppressed (Ugwu et al., 2007). Effective mixing can decrease the DO in cultures and provide a good mass transfer of O_2 and CO_2 in the culture system, thus enhancing heat transfer (Chisti, 1989; Jhawar et al., 2014). Therefore, growth rate of algae is limited by hydrodynamic stresses generated by different bioreactor configurations (Hodaifa et al., 2010; Suzuki et al., 1995).

Gas hold-up is a significant factor in PBR design and strongly affect the reactor performance. It is the volume ratio of the gas phase in the distribution between gas and liquid phases or the residence time of the gas in the

liquid. Given that aeration can occur in the riser, gas hold-up in the riser should be higher than the downcomer (without aeration) to create a liquid circulation pattern (Blanco et al., 2013; Molina et al., 2001; Wang et al., 2012). The design volume of the reactor and the gas-liquid contact area for mass transfer also depend on the gas hold-up, which is related to the bubble size and gas-liquid interfacial area for mass transfer. Thus, the gas-liquid interfacial area is based on the liquid volume or gas- liquid dispersion volume (Blanco et al., 2013; Bitog et al., 2011; Posten, 2009). Mixing time is defined as the time for a point addition to the vessel to distribute uniformly and is related to gas hold-up (Fu et al., 2003; Posten, 2009). Therefore, the rapid and homogeneous distribution of the medium and the gas provided are important factors in determining the PBR performance. Reactor type, draft tube diameter and reactor height are also significant parameters affecting the mixing performance (Chisti, 1989; Dursun and Akosman, 2006).

The aim of this study is to evaluate and optimize the performance of different column PBRs for the cultivation of *Chlorella vulgaris* and lipid production. The effects of PBRs configuration (i.e. reactor types, inner-tube lengths and bubbling flowrates) on the biomass production and lipid accumulation were also investigated.

Materials and Methods

Microalgae culture

Local freshwater microalgae, *Chlorella vulgaris*, isolated from Nam Sang Wai in Hong Kong were selected to used in this study based on the faster growth rate and higher biomass production found in our previous studies (Wong et al., 2012a; 2012b; 2012c; 2014; 2015a). The thicker cell wall of *Chlorella vulgaris* is also favourable to prevent cell lysis due to agitated mixing in PBRs. *Chlorella vulgaris* were cultivated with BG11 medium and ambient air at a constant temperature of 25℃ in PBRs in triplicate. Light was provided by cool-white fluorescent lamps at 9000 lux with a dark/light cycle of 16:8 h for 14 days.

Experimental Design

A column PBR with a capacity of 16 L was fabricated using transparent acrylic materials. The schematic diagram of the experimental set-up is shown in Figure 1a. The dimensions of the column PBR were 60.0 cm (height) × 20.0 cm (diameter), with openings at the top and bottom sides. The thickness of the column wall was 0.3 cm. The perforated pipe sparger was located 3 cm from the bottom of the reactor with the supply of ambient air at the flowrates from 1 L/min to 6 L/min. For the transparent internal loop air-lift PBR and porous air-lift PBR, both draft tube vessels were inserted into the center of the reactor column (Figure 1b). The draft tube was located 2.0 cm from the bottom of the reactor. Two types of draft tubes with dimensions of 50 cm (height) × 11 cm (diameter) and 35 cm (height) × 11 cm (diameter) were used to evaluate the effects of draft tube length on biomass production (Figure 1c). The cross-sectional area ratio of riser to downcomer was 0.067 for both draft tube vessels. Twenty holes of 0.5 cm diameter were drilled onto the porous air-lift draft tube. A synthetic culture medium was used, and air flowrate was 1 L/min. The initial cell density was 2.1×10^6 cells/mL. For the study of the bubbling flowrate effects, three identical bubbling PBRs with various air flowrate were used to optimize the cell growth condition (Figure 1d). Three colours internal LEDs light, including red, blue and white were built in the inner tube

for illumination (Figure 1e).

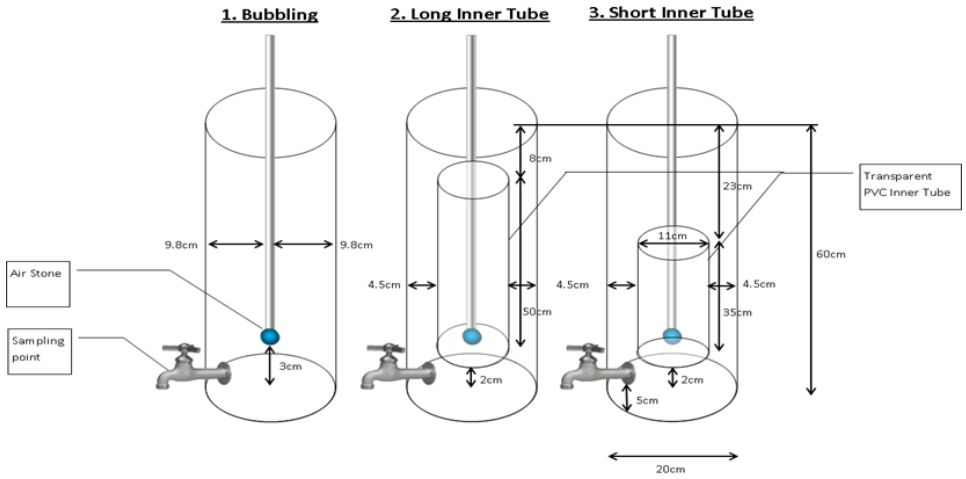

Figure 1a. Schematic diagram of the experimental set-up

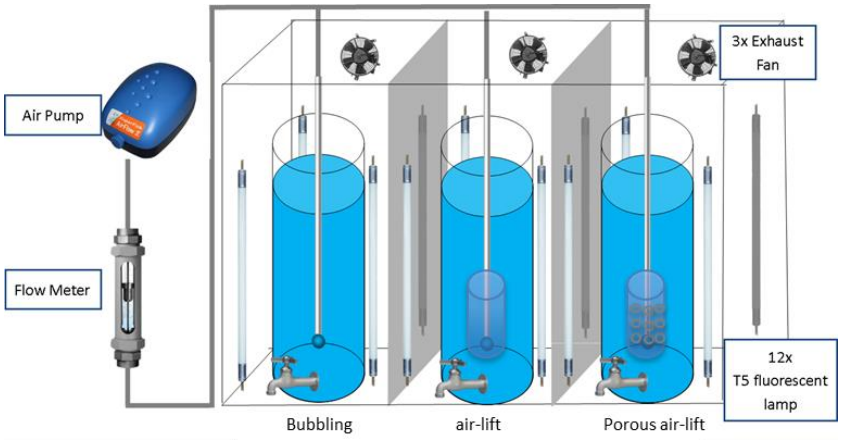

Figure 1b. Experimental set-up used to determine the effects of reactor configuration

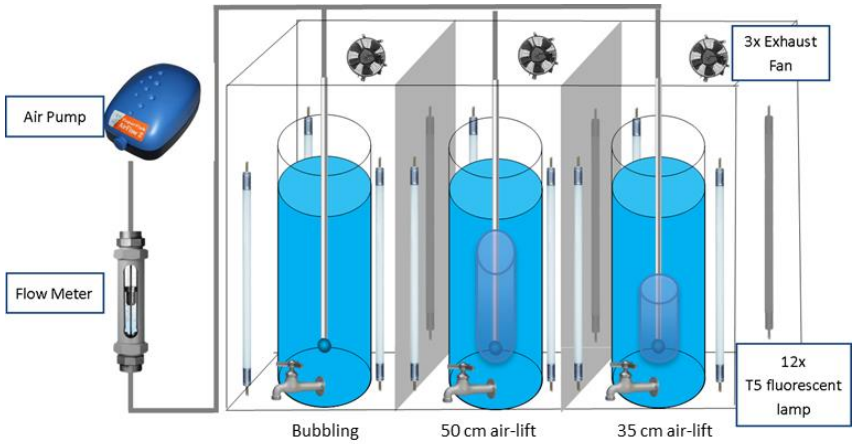

Figure 1c. Experimental set-up used to determine the effects of inner-tube length

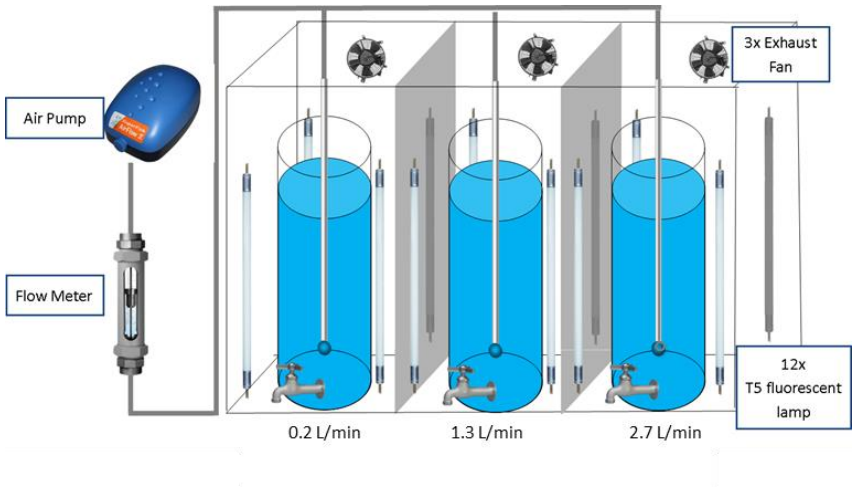

Figure 1d. Experimental set-up used to determine the effects of bubbling flowrate

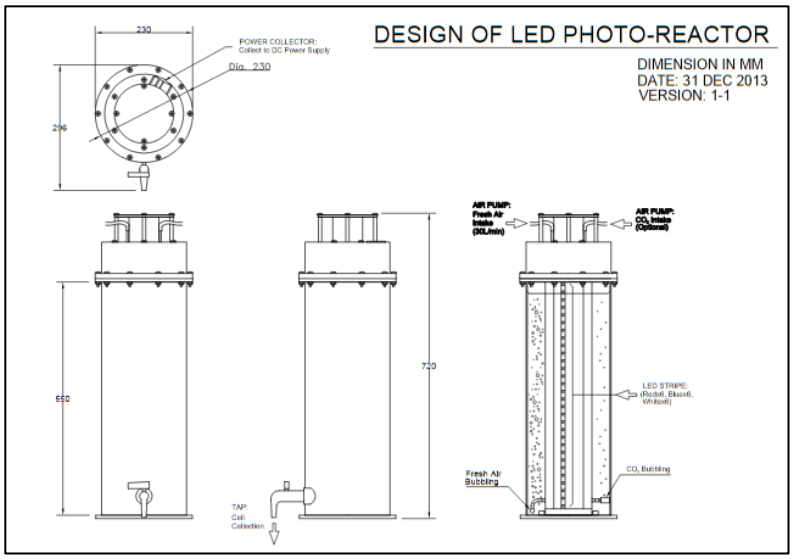

Figure 1e. Experimental design of led photobioreactor

Analytical Methods

Algal cell growth was measured by optical density (OD) at 685 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu) and the specific growth rate μ (per day) was calculated by using the following equation (Wong et al., 2015b):

$$\mu = \frac{\ln(X_1 - X_0)}{t_1 - t_0},$$

where X_1 and X_0 are the final and initial biomass concentrations (g/L) on days t_1 and t_0, respectively. The density of algal cells (cells/mL) was counted by the Sedgewick-Rafter chamber under a light microscope (BA210, Motic). The sample was diluted to appropriate concentrations of ×10, ×100, or ×1000. The final volume of the sample was 1.2 mL with one drop of Lugol's solution. Cell concentration was determined using a 10x lens with the following equation: Cell Concentration = Total number of cells x (20/1.2) x 100 (Wong et al., 2015b). Samples were collected every day for physical and chemical analyses. pH, ammonia nitrogen, nitrate nitrogen, nitrite nitrogen,

and orthophosphate were analysed according to Standard Methods (APHA, 2005). Acid-base decolourization method was used to measure the mixing time by adding 1 M HCl or 1 M NaOH to the sucrose solution in the reactor and phenolphthalein was the indicator. The velocity in the riser and the downcomer was determined by measuring the time taken by the purple colour front, developed on adding 0.5 mL NaOH to the reactor, to travel a certain distance (0.35 m) within the riser or the downcomer. The mixing time was measured by adding 0.5 mL HCl to the reactor and recording the time taken for complete decolourization of the reactor (Chisti, 1989; Molina et al., 1999). The overall gas hold-up in the air-lift PBRs was measured by volume expansion method (Chisti, 1989). The percentage change of the volume of aerated liquid was compared to the volume of gas free liquid. The variation of the liquid volume was measured by observing the heights of the surface of aerated and gas free liquids. The gas hold-up (ε) was calculated using the following equation (Aljabbar, 2010; Molina et al., 1999):

$$\varepsilon = \frac{(h_D - h_L)}{h_D};$$

where h_D is the gas-liquid dispersion height (cm) and h_L is the height of gas free liquid (cm).

For the light source of the photo-bioreactor, the Light-emitting diodes (LEDs) were of small chip size than traditional artificial light source and which could fit into the PBRs for algal biomass cultivation. On the other hand, LEDs had 941% longer life-expectancy, 500% stronger intensity, higher conversion efficiency and tolerance for switching on and off, and lower heat dissipation which was economic efficiency to cell cultivation (Matthijs, 1996; Wang et al, 2007). Narrow light emission spectra could be provided by LEDs between 20 and 30nm, thus each of the microalgae species could grow in its own optimum wavelengths, and each of the wavelengths were effects on the growth rate, such as the blue LED and red LED gave adsorption wavelength around 440-470nm and 650-680 nm, and past research showed that the blue light illumination LED increased cell size and cells grown, and red light was active divisions in small-sized cell, cell concentration and cell mass concentration (Chen et al., 2011; Kim et al., 2014; Koc, 2013; Shu, 2011; Ugwu, 2008; Vunjak-Novakovic, 2005). The different wavelength of light influenced the microalgae growth, regulation of key enzyme that associated with photosynthesis and product formation could be enhanced under these two specific light wavelengths. At the same total light intensity, 50% of the electricity consumption could be reduced by using LED light source instead of fluorescent lamps (Chen et al., 2011; Shu, 2012).

Results and Discussion

Effects of inner-tube configuration of the reactor on biomass and lipid production

Three reactor types, including bubbling, air-lift (35 cm draft tube) and porous air-lift (35 cm draft tube), were selected to investigate the effects of configuration on both biomass production and lipid accumulation. As shown in Figures 2 and 3, the highest biomass concentration in the bubbling PBR was 0.78 g/L (12×10^6 cells/mL). Given the short mixing time, the bubbling PBR provided an optimal condition for algal cells cultivation. According to Chisti (1989), the air-lift reactor with a draft tube can enhance the mixing effect and create a regular flow pattern in the riser and downcomer. In addition, the porous draft tube in the PBR can induce more small currents that shorten the mixing time. The quantities of biomass produced in the air-lift and porous air-lift PBRs were similar and comparatively lower than that of bubbling PBR (Figures 2 and 3). This finding indicates that the effect of pores on the draft tube was insignificant for microalgae cultivation in this study.

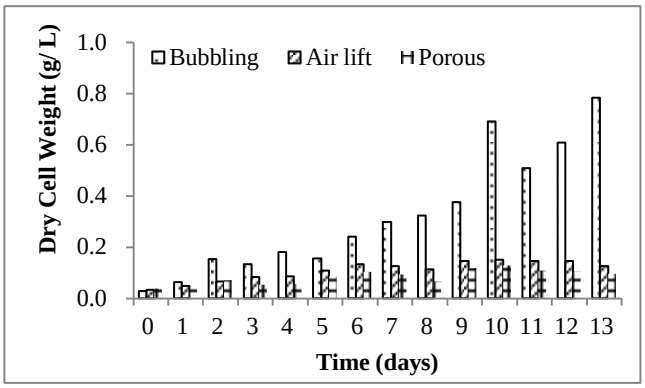

Figure 2. Biomass concentriaotn in different PBR designs

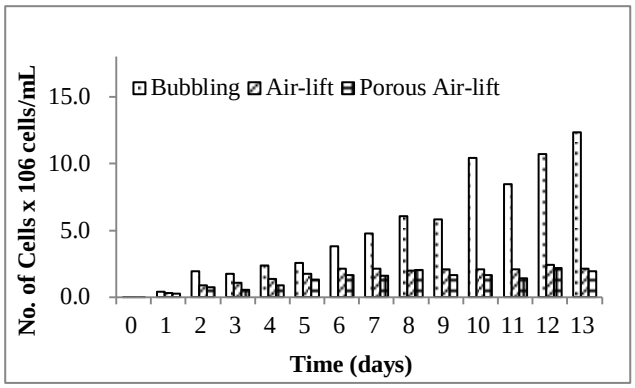

Figure 3. Growth pattern of *Chlorella vulgaris* in different PBR designs

As shown in Table 1, a negative correlation relationship between biomass production and performance of mixing time was observed. The longest mixing time was in the porous air-lift PBR, however, the lowest biomass concentration (dry weight = 0.095 g/ L) was obtained. The mixing time of bubbling PBR was 17.5 s with the highest biomass production (dry weight = 0.78 g/ L). The results also indicated that the highest lipid content (47.8%) was in the porous air-lift PBR with the lowest biomass production whereas the lowest lipid content (38.9%) was in the bubbling PBR with the highest biomass production. A negative correlation was observed between biomass production and lipid content. Therefore, the performance of different reactors was determined by the mixing time. The lipid content was enhanced by the turbulence created from the pores of the inner-tubes in porous air-lift PBRs because the stressed growth conditions was induced due to the non-uniform mixing of nutrient in porous air-lift PBRs.

Table 1. Biomass production, mixing time, lipid content in different PBR designs

Reactor type	Biomass production (g/L)	Biomass productivity (g/L/d)	Mixing time (s)	Lipid content (%)
Bubbling	0.783	0.054	17.5	39.8
Air-lift	0.126	0.007	27.8	39.8
Porous air-lift	0.095	0.004	31.7	47.8

Considering that the length of the draft tube was 35 cm (which was 20 cm shorter than the water level), a similar increasing trend from 0 to 0.02 of the gas hold-up for all tested PBRs was found at the gas flowrates less than 4 L/min (Figure 4). When the aeration flowrate was 4 L/min, the gas hold-up of the bubbling PBR exponentially increased. A linear positive correlation ($R^2 = 0.9969$) between flowrate and gas hold-up in the bubbling PBR was also observed. For the air-lift and porous air-lift PBRs with a draft tube setup, the bubbles were dispersed along the 35 cm draft tube and quickly diffused out after leaving the draft tube, which caused the gas hold-up to increase sharply from 0.02 to 0.09.

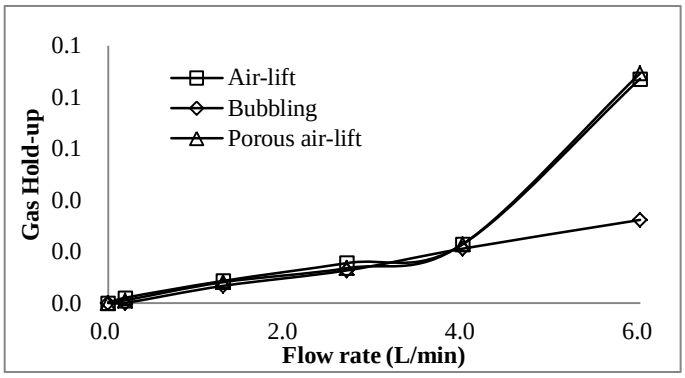

Figure 4. Gas hold-up in different PBR designs

Effects of inner-tube length on biomass and lipid production

In this study, 35 cm and 50 cm inner-tubes in the air-lift PBRs were selected to investigate the effects of the length of the inner-tube on biomass production and yield of lipid content. The bubbling PBR was used as the control. After 14 days cultivation, the cell concentration in the bubbling, 35 cm air-lift and 50 cm air-lift PBRs was 14×10^6, 11×10^6 and 7×10^6 cells/mL, respectively. The growth characteristic of the air-lift PBR with 35 cm inner-tube was similar to that of bubbling PBR (Figure 5). As shown in Table 2, the shorter the mixing time was, the better the biomass production became. The biomass production in the 35 cm air-lift PBR was 23.5% higher than that in the 50 cm air-lift PBR. This may be due to the mixing time of the 50 cm air-lift PBR was 56.9% higher than that of the 35 cm air-lift PBR. However, the biomass production of bubbling PBR was significantly higher than that of air-lift PBRs (64.8% to 115.4%). This condition can be attributed to a higher chance for the algal cell to assimilate nutrients because of better mixing (Barbosa et. al., 2003). Good mixing could also prevent the settling of algal cells to maintain biomass in suspension, and absorb more light for photosynthesis, resulting in higher productivity.

Table 2. Biomass production, mixing time, biomass productivity in different inner-tube lengths

Reactor type	Biomass production (g/L)	Biomass productivity (g/L/d)	Mixing time (s)
Bubbling	0.842	0.049	17.5
35 cm air-lift	0.518	0.026	24.5
50 cm air-lift	0.398	0.018	53.5

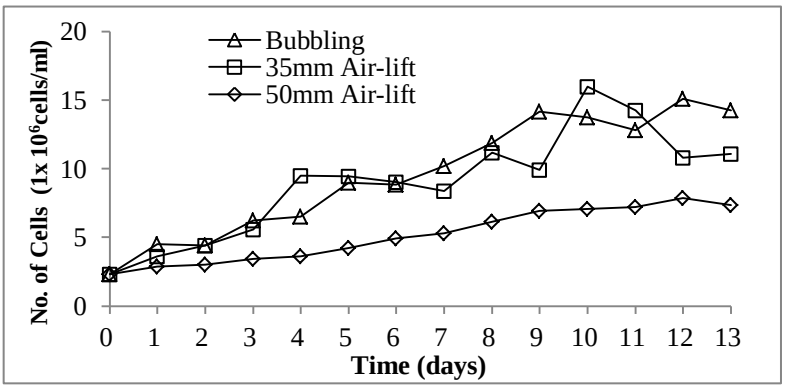

Figure 5. Growth pattern of *Chlorella vulgaris* in air-lift reactors with various inner-tube lengths

The inner-tube in the PBR induces a regular flow pattern with slower velocity compared with the bubbling PBR. Table 3 shows that the biomass was significantly attached on the inner wall of the air-lift PBRs, comparing with the bubbling PBR. It may be due to once the flow in the air-lift PBRs was equilibrated; the microalgae easily attached to the inner wall surface and became thicker. Considering that the bubbling PBR induced a random flow pattern, a higher biomass production was achieved in the bubbling PBR because the microalge are much more difficult to attach to the inner walls. However, the lipid content (~30-40%) was not significantly affected by the increase in inner-tube length because lipid accumulation is mainly enhanced by stress conditions.

Table 3. Biomass production of suspended and attached samples

Reactor type	Suspended (g/L)	Attached (g/L)
Bubbling	0.84	3.22
35 cm Air-lift	0.52	8.44
50 cm air-lift	0.40	8.07

As shown in Figure 6, the gas hold-up of the bubbling PBR showed a positive linear correlation ($R^2 = 0.9969$) with an increasing flowrate from 0 L/min to 6 L/min. The trend of gas hold-up of the 35 cm air-lift PBR was similar to that of the bubbling PBR, especially at the flowrate 1.5 L/min to 6 L/min. This result can be attributed to the bubbles being centrally-dispersed within the shorter (35 cm) draft tube with an 11 cm diameter. However, the gas hold-up of the 50 cm air-lift PBR exponentially increased at the flowrate below 1 L/min, and then linearly increased from 1 L/min to 6 L/min. It may be due to the bubbles being centered within the longer (50 cm) inner-tube. Once the bubbles diffused out of the inner-tube, they caused the gas hold-up to increase and obtained the highest gas hold-up value (0.04) at the flowrate of 6 L/min.

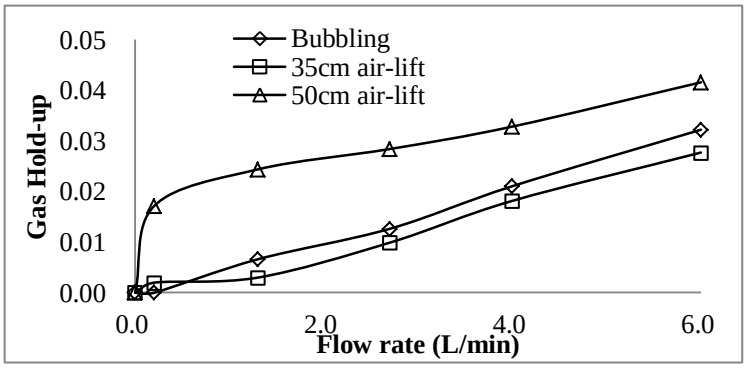

Figure 6. Gas hold-up in reactors with various inner-tube lengths

Effects of bubbling flowrate on biomass and lipid production

Three identical bubbling PBRs with the flowrates of 2.7 L/min, 1.3 L/min and 0.2 L/min were used to investigate the effects of flowrate on biomass production and lipid yield. The bubbling PBR with the highest flowrate (2.7 L/min) produced the highest biomass yield (16×10^6 cells/mL), whereas the PBR with the flowrate of 0.2 L/min was not successful to produce biomass (Figure 7). It may be due to higher flowrate provides better mixing effects in the bubbling PBR, which not only prevents microalgae settling but also absorbs more light energy for photosynthesis to have higher biomass productivity.

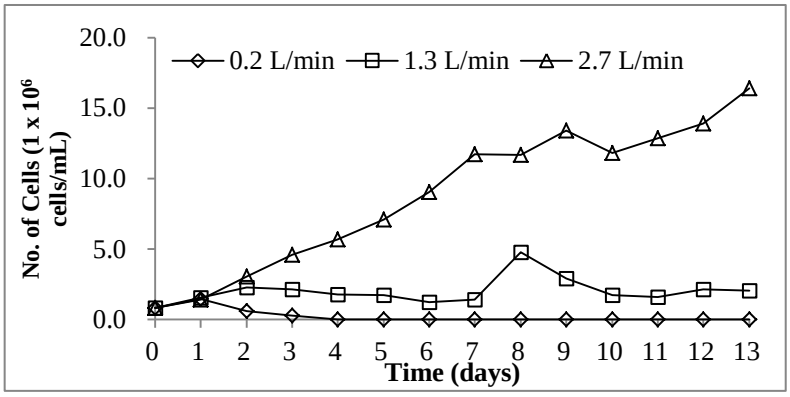

Figure 7. Growth pattern of *Chlorella vulgaris* in bubbling PBRs under various flow rates

Samples were taken every six hours for first fourth days to analyze the change in biomass concentrator (Figure 8). In the case of high flowrate (2.7 L/min), certain biomass loss was observed during night time because a portion of the intracellular carbohydrate was consumed by respiration. Given the absence of a light source, photosynthesis also stopped and no carbohydrate was produced. In the presence of a light source, the biomass continuously increased as the photosynthesis rate was faster than the respiration rate. Under the lower flowrates (i.e., 0.2 or 1.3 L/min), the biomass increased in the first two days and then decreased significantly due to the turbulence caused by aeration (bubbling) was not enough to resist the auto-flocculation of the microalgae and provided poor nutrient mixing for cell cultivation. The results are consistent in the findings obtained in our previous studies (Wong et al., 2014; 2015b).

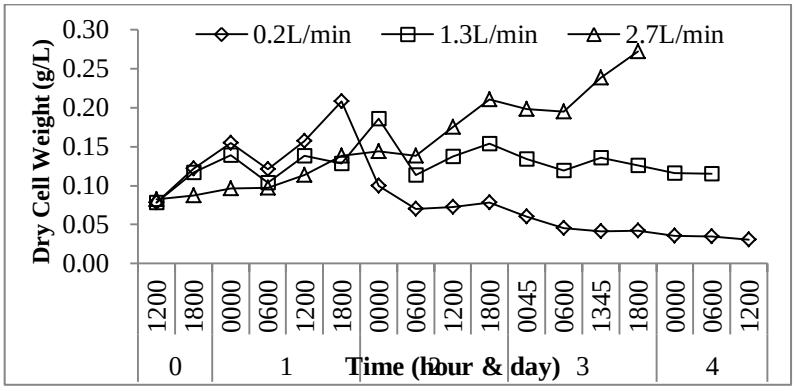

Figure 8.Biomass concentration in bubbling PBRs under various flow rates

After 14-d cultivation, the bubbling PBR with the flowrate of 2.7 L/min produced the highest biomass production (0.75 g/L) at the fastest mixing time (18 s) (Table 4). Similar to previous cases, a negative correlation was also observed between biomass production and mixing time. The shorter the mixing time was, the higher the biomass production became. However, the highest lipid content of *C. vulgaris* (28%) was successfully produced in the bubbling PBR with the flowrate of 1.3 L/min (Table 4). These results indicated there is no strong correlation between lipid content and biomass productivity in bubbling PBRs.

Table 4. Biomass production, mixing time, lipid content in bubbling PBRs with various air flowrates

Flowrate (L/min)	Biomass production (g/L)	Biomass productivity (g/L/d)	Mixing time (s)	Lipid content (%)
0.2	0.04	<0.001	41.3	<0.01
1.3	0.15	0.005	20.5	28.0
2.7	0.74	0.048	17.5	19.2

Effects of LED light colors on biomass and lipid production

After 10-d cultivation, the biomass production was an increasing trend for both of the light source from initial to Day 7. The biomass production in Day 10 in white LED was the highest (1.25 g/L) while blue LED, red LED and open pond were 0.80 g/L, 0.35 g/L and 0.58 g/L respectively (Figure 9). It showed that the biomass production in white LED photo-bioreactor was double than the open pond, which was similar results (Jorquera et al, 2010). Moreover, after Day 3, there was a significant difference in biomass production between open pond and Photo-bioreactor_white and three colours of photo-bioreactor ($P<0.05$) (Table 5). For the maximal biomass production, the white LED photo-bioreactor was 1.57 times more than blue LED photo-bioreactor, and then was red LED photo-bioreactor (2.21 times), and lastly was open pond (3.38 times).

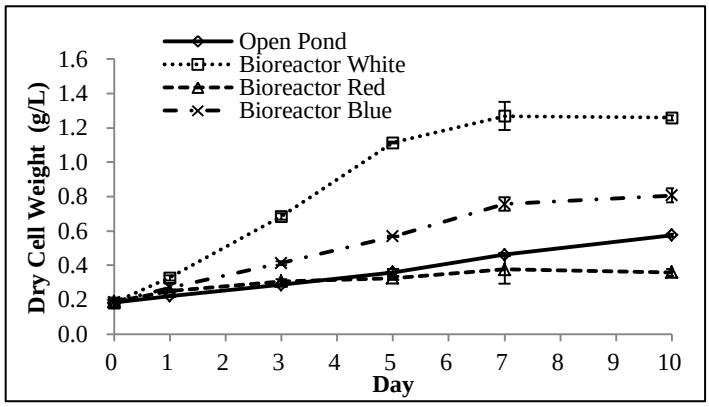

Figure 9 Biomass concentration in Open Pond (-◊-), Photo-bioreactor_white (-□-), Photo-bioreactor_red (-Δ-), and Photo-bioreactor_red (-×-)

Table 5 Effects of types of cultivation methods on biomass concentration

Dry Biomass	SS	*df*	F	P-value
(i) Open pond vs Photo-bioreactor_white	0.629	1	5.000	0.049
(ii) Photo-bioreactor White vs Red vs Blue	0.777	2	3.895	0.043

The Lipid content was no significant difference between different LED light color conditions ($P>0.05$) (Table 6), while the lipid productivity was significant difference in these three light color conditions (Table 6). Also, Figure 10 showed that the Photo-bioreactor_white had the highest value of lipid content and lipid productivity. Moreover, the photo-bioreactor under white light condition had a maximum amount (0.28g/L/day) of the lipid productivity and also of the higher of the average lipid productivity (0.12g/L/day) which was shown in Table 7. The maximum of lipid content showed in the white LED photo-bioreactor was 1.64 times higher than the open pond, and then was blue LED photo-bioreactor (2.21 times), and lastly was red LED photo-bioreactor (2.46 times). For The

maximum of productivity, the white LED photo-bioreactor was 4.37 times higher than the open pond, and then was blue LED photo-bioreactor (6.53 times), and lastly was red LED photo-bioreactor (9.60 times).

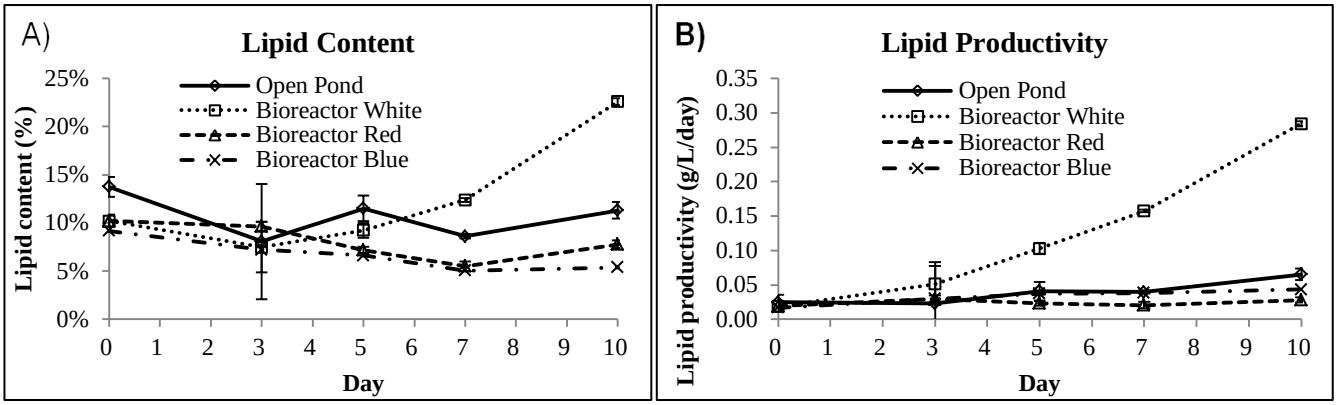

Figure 10 A) Lipid Content, and B) Lipid Productivity of the the Open Pond (-◊-), Photo-bioreactor_white (-□-), Photo-bioreactor_red (-Δ-), and Photo-bioreactor_red (-×-)

Table 6 Effects of types of cultivation methods on A) Lipid content, and B) Lipid productivity.

A) Lipid content	SS	*df*	F	P-value
(i) Open pond vs Photo-bioreactor_white	0.001	1	0.369	0.560
(ii) Photo-bioreactor White vs Red vs Blue	0.009	2	3.164	0.079

B) Lipid productivity	SS	*df*	F	P-value
(i) Open pond vs Photo-bioreactor_white	0.017	1	3.159	0.113
(ii) Photo-bioreactor White vs Red vs Blue	0.029	2	4.051	0.045

Table 7 Maximum and average biomass, lipid content and lipid productivity

Condition	Biomass productivity (g/L/day)		Lipid content (%)		Lipid productivity (g/L/day)	
	Max.	Avg.	Max.	Avg.	Max.	Avg.
Open Pond	0.58	0.35	13.76	10.65	0.07	0.04
Photo-bioreactor white	1.27	0.81	22.58	12.39	0.28	0.12
Photo-bioreactor red	0.38	0.30	10.22	8.08	0.03	0.02
Photo-bioreactor blue	0.81	0.50	9.16	6.69	0.04	0.03

Conclusion

The results of current study indicate that the algal biomass production of *Chlorella vulgaris* in PBRs was mainly affected by the reactor configuration. The bubbling PBR was the better choice for the cultivation of *Chlorella vulgaris.* However, the lipid content was enhanced by the turbulence created from the pores of the inner-tubes in porous air-lift PBRs because the stressed growth conditions. The results indicate that the air-lift PBRs with shorter draft tubes (35 cm) were better than the PBRs with longer draft tube (50 cm) for the

performance of biomass production. The higher flowrate (2.7 L/min) of air supply in bubbling PBRs can cultivate higher biomass production but produce lower lipid content due to the shorter mixing time. The photobioreactor under white LED light condition was the best for biomass and lipid production.

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