# RESEARCH Open Access

# Cultivation of *Nannochloropsis* sp. using narrow beam angle light emitting diode in an internally illuminated photobioreactor

Mohamad Taisir<sup>1</sup>, Chee Loong Teo<sup>2</sup>, Ani Idris<sup>2\*</sup> and Affendi M. Yusuf<sup>1</sup>

# **Abstract**

**Background:** This paper reports on the growth condition of *Nannochloropsis* sp. in an annular column-type photobioreactor (PBR) using light-emitting diode as an internal illumination.

**Methods:** The microalgae growth in the 20-L batch culture mode under mixed blue (450 nm) and red (660 nm) light-emitting diode (LED) in various conditions such as photoperiod and light intensity (controlled by supplied current) was monitored. Compact-type 5-W LED module with narrow beam angle (radiation pattern) was installed in the PBR so as to obtain higher intensity and deeper penetration to the culture.

**Results:** Based on the PBR dimension with optical path length 120 mm, the minimum light intensity required at the PBR tank inner surface at initial stage of cultivation was approximately 350–370 mol m $^{-2}$ s $^{-1}$ , while mean light intensity derived was 140–160 mol m $^{-2}$ s $^{-1}$ . Photoperiod ratio of light:dark at 18:6 h provided better results compared to 12:12 in terms of final cell density achieved. Efficiency of light utilization was calculated to be 9.0 × 109 cell/mol photon (0.49 g/mol photon), while biomass volumetric productivity was 0.04 g L $^{-1}$ day $^{-1}$ .

**Conclusion:** The usage of narrow beam angle LED was feasible to be used but with further improvement is necessary.

**Keywords:** LED, Photobioreactor, Microalgae, Biodiesel

# **Background**

Microalgae have an outstanding performance compared to the terrestrial plants based on its high multiplication rate and high lipid content (Rawat et al. 2013). One of its potential is to be used as biomass source for biodiesel production (Chisti 2007). Although successfully developed, open pond cultivation method which generates low density culture is faced with a tough situation to meet the low price requirement of biodiesel. In recent years, the trend of mass cultivating microalgae has shifted from open pond system to closed photobioreactor (PBR) system. PBR technology has attracted the attention of industrial and scientific communities observed

by the increasing numbers of scientific research papers published over the past 30 years (Olivieri et al. 2014). Among the reasons for this shift are to reduce contamination problem and achieve higher cell density. Nevertheless, issues such as temperature rising, inconsistent solar rays, weather fluctuation, photoinhibition due to excessive light intensities, night time biomass losses are among problems that remain to be improved in the outdoor PBR.

In regards to light parameters, indoor PBR using artificial lights may offer some solutions to overcome the problems in the outdoor PBR which depends to sunlight. With rapid development of highly energy efficient LEDs which are increasingly cheaper and consume less energy than ordinary lamps, the potential of using artificial lights in PBR and its economic feasibility need to be reviewed (Fu et al. 2012). Consequently, the cost and energy consumption are factors that need to be seriously considered

<sup>&</sup>lt;sup>2</sup> Department of Bioprocess Engineering, Faculty of Chemical & Energy Engineering, c/o Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, UTM Skudai, 81310 Johor Bahru, Johor, Malaysia Full list of author information is available at the end of the article



<sup>\*</sup>Correspondence: ani@cheme.utm.my

(Blanken et al. 2013). It seems that the high cost of PBR with artificial lights may not be an appropriate solution at this moment, but researchers are continuing to study and improve it as there are potentials to develop it as a standalone production system or as inoculation system for various applications.

Nannochloropsis sp. is among the marine microalgae that has been widely studied in aquaculture sector (Alsull et al. 2012; Bong et al. 2013), and currently being actively researched for biodiesel production worldwide (Quinn et al. 2012; Pegallapati and Nirmalakhandan 2013). The main pigment for photosynthesis in Nannochloropsis sp. is *chlorophyll a*, while *chlorophyll b* and *c* are completely lacking. In relation to this, light quality plays an important role in photoautotrophic cultivation of microalgae. Photosynthetic pigments such as chlorophylls are highly react to a particular light spectrum bandwidth; such as blue at 450-475 nm and red at 630-675 nm (Richmond 2004). Previous studies on a lab scale have identified the potential of blue and red LED as artificial light source for microalgae growth (Teo et al. 2014a, b). Similar findings revealed that optimum growth of C. vulgaris (Atta et al. 2013) and Nannochloropsis sp. (Ra et al. 2016) were obtained when cultivated in blue LED light, while other suggested the usage of red LED light as the optimum wavelength for Chlorella sp. growth (Yan et al. 2016). In addition, yellow light with supplemental of minimal blue light was found to be effective to maximize Chlamydomonas reinhardtii productivity by lowering the biomass specific light absorption rate (de Mooij et al. 2016). Variations of lighting strategies, PBR volume and configurations made it difficult to determine the basic method of choosing the best lighting parameters, although it is clear that monochromatic light especially blue and red provide better performance than wide spectrum light in general energy consumption.

Another critical parameter is light intensity (quantity), whereby it varies with its position and time in the cultivation process of microalgae (Huang et al. 2012) due to the nature of microalgae as an efficient light receiver. In lab scale experiment with small volume of culture and short light path, incident light intensity is commonly used as the light quantity parameter to determine the optimum condition (Wahidin et al. 2013; Teo et al. 2014b). However, in up-scale or large volume cultivation, mean light intensity is a better indication of light condition; light intensity at the illumination surface, *IS* and opposite surface, *BS* (end of light path) need to be considered (Ogbonna and Tanaka 2000). Light distribution inside the PBR is very important to ensure light energy to biomass conversion is at optimum condition.

The common approach in utilizing sunlight receiving for an outdoor PBR is to design it with high surface/

volume ratio (SVR) (Posten 2009). Tubular and flat panels PBR have good properties in terms of high SVR for utilizing solar rays (Grima et al. 1996; Zijffers et al. 2010; Silva Benavides et al. 2013), while bubble column is not widely preferred. However, when considering artificial light as the light source for PBR, huge amount of energy would be needed to achieve intensity and uniform distribution of light similar to the sunlight. We are suggesting that this approach need to be reviewed for indoor application. As an example, LED is usually mounted in a closely packed array form to illuminate the flat panel PBR surface (Lee and Palsson 1994). In this case, the intensity of standard LED chip with wide angle (110°-120°) may not be sufficient to penetrate culture depth of more than 20 mm, so a PBR with short optical path length would be preferable. Yet, it would be difficult to illuminate the whole panel surface as PBR volume increase (up-scale project). So, an alternative method which is suitable for up-scale PBR is necessary, while at the same time there must be consideration for practical light quantity in high volume cultivation.

In general, bubble column has the advantage for upscale project as it can easily accommodate large volume, efficient contact between the gas and liquid phases, relatively low cost for setup and operating, well accepted for other industrial usage and minimal maintenance is required. Although it is not popular as an outdoor PBR, there is potential to develop it as an indoor PBR with artificial light. In regards to microalgae cultivation in bubble column as indoor type, internal illumination method was found to be better in efficiency compared to external illumination (Pegallapati et al. 2012). This approach would also facilitate the daily operation in monitoring the microalgae growth since the lightings are placed internally. Few types of lightings have been proposed before such as using fluorescent light, optical fibre and others (Ogbonna et al. 1996; Csogor et al. 2001; Suh and Lee 2001; Xue et al. 2013). Light conversion efficiency is the main concern for PBR with artificial light, however due to varieties in each artificial light optical properties, it is quite difficult to have an equal comparison. The balance of microalgae growth and lightings energy, lightings layout, microalgae cell density are among parameters that need to be considered when using the artificial lights in PBR.

Therefore the aim of this study is to evaluate the performance of *Nannochloropsis* sp. cultivation in an internally illuminated PBR with LED. It is a primary scale-up (20 L) from previous lab study (0.5 L) which used red and blue LED to cultivate microalgae. The use of narrow beam angle LED was proposed in order to penetrate the deep culture (120 mm) in the bubble column, as normal wide angle LED has limitation in this aspect. The experimental data obtained can be utilized for further scale up (100 L) of an internally illuminated PBR using LED in future work.

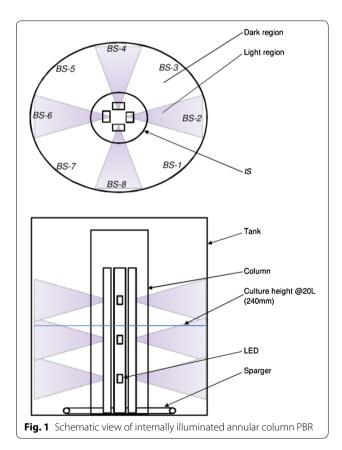
#### Methods

#### **LED** luminaire

The LED luminaire consisted of four LED bars mounted together to top and bottom plate. The LED bar was installed with three units of 5 W LED module, each package contained a total of 16 LED chips with combination of blue 450 nm and red 660 nm in quantity ratio 8:8 and intensity ratio approximately 60:40 %. The overall optical beam angle was designed to be at 55° (narrow angle) to achieve high light intensity through the built-in lens design. The LED luminaire was powered by using either adjustable current supply (200–340 mA) or a constant current driver (350 mA) throughout the experiments. The heat from LED was dissipated through the bar itself which is fabricated from aluminium material.

# Photobioreactor (PBR)

The PBR tank was made of stainless steel with internal diameter 380 mm and height 440 mm as depicted in Fig. 1. A column made of acrylic material with outer diameter of 140 mm was fixed at the center of tank to provide space for LED luminaire. Circular sparger (connected to an air pump) with eight holes of diameter 2 mm was placed at the bottom of tank to provide the ambient air for mixing purposes and  $\mathrm{CO}_2$  source. For



microalgae cultivation in large volume such as  $20\,L$ , there is no necessity to perform sterilization (Hannon et al. 2010), therefore only basic cleaning was done and  $70\,\%$  alcohol was sprayed on the surface of PBR a day before running the experiment. The high density inoculation allows microalgae population to expand rapidly, minimizing end-product loss due to contamination.

# **Culture and medium**

Nannochloropsis sp. strain was originally obtained from the culture collection of Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah, Malaysia. The Nannochloropsis sp. cells were cultured in sterilized seawater (autoclaved at 1–2Bar, 121 °C) and enriched with Walne's medium which contains: 100 g NaNO<sub>3</sub>, 1.3 g FeCl<sub>3</sub>·6H<sub>2</sub>O; 0.36 g MnCl<sub>2</sub>·4H<sub>2</sub>O; 33.6 g H<sub>3</sub>BO<sub>3</sub>; 45 g Na<sub>2</sub>·EDTA; 20 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O; 2.1 g ZnCl<sub>2</sub>; 2 g CoCl<sub>2</sub>·6H<sub>2</sub>O; 0.9 g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O; 2 g CuSO<sub>4</sub>·5H<sub>2</sub>O; 0.001 g Vitamin B<sub>1</sub> and 0.2 μg Biotin per liter.

# **Experiment setup**

Each experiment was conducted in a 20 L working volume using batch culturing method. Experiment 1 was conducted at regulated current between 200 and 340 mA (an increment of 20 mA in every 2 days) with maximum intensity of 200–220  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> at BS, photo period of 12:12 h (light:dark) and initial cell density of  $2.7 \times 10^6$  cell/mL (3.59 g/L). Experiments 2 and 3 were conducted by providing each LED package with a constant current of 350 mA with maximum intensity of 350-370 µmol m<sup>-2</sup>s<sup>-1</sup> at BS, while photo period was set at 12:12 and 18:6 h respectively. Initial cell density for experiment 2 and 3, was reduced to within  $1.9-2.0 \times 10^6$ cell/mL (2.52-2.66 g/L), after we realized that margin of light intensity was not sufficient to be increased more due to LED current at 500 mA causing heat issue for this prototype.

# Measurement of photosynthetic photon flux density (PPFD)

Optimum light intensity is critical for the growth of microalgae, but the attenuation of light is very much influenced by the culture depth. Light intensity or PPFD was measured using a quantum sensor connected to Light Scout Dual Solar quantum light meter, in unit of µmol m<sup>-2</sup>s<sup>-1</sup>. PPFD evolution of the light intensity was confirmed by taking measurements at the illumination surface (outer surface of internal acrylic column), *IS* until opposite surface (inner surface of PBR tank), *BS* at incremental distance of 15 mm and fix height (highest light intensity point). Due to quantum sensor probe size/height (from base to sensor surface) itself is 15 mm,

the PPFD measurement at inner surface BS was represented by the distance at 105 mm, instead of 120 mm. Throughout the experiments, PPFD at BS was monitored every 2 days at eight equally spaced radial locations. Each four points were representing the intensity of light region (LED beam covered region) and dark region (adjacent region where LED beam was limited) respectively. Since the LED module radiation pattern was narrow in angle (focus effect), the boundary of light and dark region can be clearly identified visually. Each LED module covered approximately an area of  $130 \times 130$  mm (0.0169 m<sup>2</sup>) square boundaries at BS, resulting total coverage of light region from four LED bar (12 modules) equal to about 40 % (0.2028 m<sup>2</sup>), while dark region about 60 % (0.3222 m<sup>2</sup>) from total PBR inner surface area which is 0.525 m<sup>2</sup>. The dark region is defined as the area which received less than 70 % of peak intensity at BS. The effect of different ratio of light/dark region was not investigated in this study based on two reasons; (1) Increasing the light/dark region may not feasible due to space constraint, LED heat issue and cost factor, (2) Decreasing the light/dark region may effect to the microalgae growth due to lack of light by reduction of SVR. Figure 1 shows the schematic view of the PBR.

# Measurement of microalgae growth and lipid content

The growth of microalgae was mainly monitored by using optical density method. Samples were taken every 2 days, and measured using a UV–vis spectrophotometer (Shimadzu UVmini-1240) at 540 nm (Rocha et al. 2003). Cell number counting was measured by a direct microscopic count with a 0.1-mm-deep Neubauer haemocytometer (BOECO, Hamburg Germany) and a light microscope (Olympus CX21, Japan). A correlation curve between optical density and cell number was used to evaluate the cell density condition throughout the experiment. The specific growth rate, μ was calculated from the Eq. (1):

$$\mu = \frac{\ln\left(N_2/N_1\right)}{t_2 - t_1} \tag{1}$$

where  $N_2$  and  $N_1$  are the cell densities at time  $t_2$  and  $t_1$  (exponential phase) respectively. The time required to duplicate the cell number; division rate, k was calculated from the Eq. (2):

$$k = \frac{\mu}{\ln 2} \tag{2}$$

The volumetric productivity,  $P_x$  (cell L<sup>-1</sup> h<sup>-1</sup>) was calculated using Eq. (3):

$$P_x = C_x \mu \tag{3}$$

where  $C_x$  (cell mL<sup>-1</sup>) refers to maximum cell density achieved from the batch cultivation.

The amount of lipid in *Nannochloropsis* sp. was determined according to the method described previously by Teo et al. (2014a) using improved Nile red staining method (Chen et al. 2011) and Perkin Elmer LS-55 fluorescence spectrophotometer. For the Nile red staining,  $50~\mu L$  of Nile red (9-diethyl-amino-5H-benzo[a]phenoxazine-5-one; Sigma, USA) in acetone representing a concentration of 0.1 mg mL<sup>-1</sup> was added to the 1 mL of sample. The mixture was pretreated using a microwave oven for 1 min. The excitation and emission wavelengths for the fluorescence were 490 and 585 nm respectively.

### Measurement of light utilization efficiency

The amount of light/photon absorbed (mol s<sup>-1</sup>),  $E_a$  is calculated using Eq. (4):

$$E_a = I_{\text{mean}} \times 10^{-6} \times A_{\text{ill}} \tag{4}$$

where illuminated area,  $A_{\rm ill}=2\pi rh, r$  is the radius of the PBR tank (190 mm) and h is the height of culture volume (240 mm). The efficiency of light utilization can be expressed as the biomass yield on light energy and  $Y_{x,E}$  is the cell quantity produced per amount of light energy absorbed (mol of photons absorbed) as shown in Eq. (5)

$$Y_{xE} = \frac{P_x \times V_{\text{PBR}}}{E_a \times 3600} \tag{5}$$

where  $P_x$  is the volumetric productivity (cell L<sup>-1</sup> h<sup>-1</sup>) and  $V_{\rm PBR}$  is the volume of PBR (L), while conversion rate of 0.0545  $\times$  10<sup>-9</sup> g/cell (Rocha et al. 2003) was used for above calculation. For reference purpose, light intensity at a point inside the culture, I can be calculated using a simplified Beer-Lambert's law equation as shown in Eq. (6)

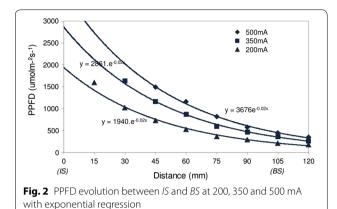
$$I = I_0 e^{-KCL} \tag{6}$$

where  $I_0$  is the incident light intensity at culture surface, K is the biomass absorption, C is the biomass concentration, and L is the length of light path.

# **Results and discussions**

# Light intensity (PPFD) inside the PBR

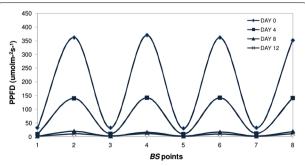
By using high power 5 W LED package, the light intensity (before the addition of culture) at IS achieved more than 2000  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, which is approximately equal to incidental sunlight intensity during sunny day in Malaysia (on horizontal surface). The light intensity at BS is approximately 200–220, 350–370, 440–460  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> respectively for supplied current of 200, 350 and 500 mA for this type of LED. However, due to high heat generated by LED when running at 500 mA, the cultivation at this parameter was omitted. In future work, the use of convection fan or modification on the size/shape of aluminium bar as heat dissipater may need to be reviewed. Figure 2



shows the PPFD evolution measured between two points, *IS* (0 mm distance) and *BS* (105 mm distance), while the value at 120 mm distance is based on calculation. The PPFD decreased as the distance increased from surface of internal column until the tank surface. The light penetration to the tank surface was further reduced by the cultivation days due to mutual shading of the microalgae as can be observed from Fig. 3, which exhibits the light distribution at the *BS* in 0, 4, 8 and 12 days of cultivation (experiment 2). Further details of relationship between PPFD and cell density will be explained in next section.

# Relationship of light intensity and photoperiod on *Nannochloropsis* sp. growth and lipid production

Figure 4 shows the cell growth profiles for each experiment. In experiment 1 (Fig. 4a), the LED current was regulated between 200 and 340 mA with incremental of 20 mA in each 2 days. This provided initial light intensity of 200–220  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> at *BS*, while the photoperiod was set at 12:12 h (light:dark). The cells remained in lag phase until 6th day, probably adapting to the lighting condition or possibly the mean light intensity was not sufficient for the culture. Exponential phase eventually can be seen between 8th and 10th day with LED



**Fig. 3** PPFD changes at *BS* (point 1–8) at 0, 4th, 8th and 12th day of cultivation period (data from experiment 2)

current at approximately 300 mA, while light intensity at BS reduced from 200-220 to 70-90  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> due to the cell started to shade the light. The culture reached the stationary phase after 12th day with PPFD recorded at 20–40  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, possibly due to the nutrient depletion. As overall, the growth rate was very low with the highest cell density recorded was  $5.0 \times 10^6$ cell/mL (0.27 g/L), while the specific growth rate, µ was 0.126 day<sup>-1</sup> (calculated between 8th and 12th day). It was suspected that the light was insufficient to be accessed by the average cells for optimum photosynthetic reaction. In experiment 2 (Fig. 4b) and 3 (Fig. 4c) with lower initial cell density, the LED current was set constant at 350 mA for higher mean light intensity compared to previous experiment. This provided initial light intensity at 350-370  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (BS), and as a result a more encouraging condition for culture growth can be seen. Moderate light intensities (400–1000 µmol m<sup>-2</sup>s<sup>-1</sup>) were reported to be suitable for microalgae growth (Yan et al. 2016). In experiment 3 with photoperiod of 12:12, the culture growth started to accelerate after the 2nd day, with PPFD recorded at 160–180 µmol m<sup>-2</sup>s<sup>-1</sup>. The cells experienced the exponential growth after 4th day, and remained in linear phase until 10th day. During this period the PPFD gradually decreased from 130–150 μmol m<sup>-2</sup>s<sup>-1</sup> to 60-80 µmol m<sup>-2</sup>s<sup>-1</sup> and finally reached to  $10-20 \mu \text{mol m}^{-2}\text{s}^{-1}$ . The maximum cell density recorded was  $6.4 \times 10^6$  cell/mL (0.35 g/L), while the specific growth rate,  $\mu$  was 0.195 day<sup>-1</sup> (calculated between 4th and 8th day). In experiment 3 with longer photoperiod of 18:6, the cells immediately entered the exponential growth phase after 2nd day with PPFD recorded at 130-150  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>. During the linear growth phase, the PPFD decreased to 10–20 μmol m<sup>-2</sup>s<sup>-1</sup>, and the growth entering stationary phase at 8th day which was earlier than previous experiments. Longer exposure to the light had possibly allowed the cells to grow faster compared to the previous experiments. The maximum cell density recorded was  $7.1 \times 10^6$  cell/mL (0.39 g/L), while the specific growth rate, μ was 0.220 day<sup>-1</sup> (calculated between 2nd and 6th day).

In these experiments, it was observed that the cell density graph intersected with PPFD graph at around 140–160 µmol m $^{-2}$ s $^{-1}$  (refer to Fig. 4a–c), which is proposed to be the mean light intensity,  $I_{\rm mean}$  (µmol m $^{-2}$ s $^{-1}$ ) of this PBR. Alternatively by using Eq. (6), where  $I_0$  is determined as 2861 µmol m $^{-2}$ s $^{-1}$  (actual measurement value exceeded the quantum meter range), K is assumed as 0.15 m $^2$  g $^{-1}$ , C is biomass concentration at the intersection graph (2.7  $\times$  10 $^6$  cell/mL or 148 g/m $^3$ ), and L at 0.12 m; light intensity, I can be calculated as 199 µmol m $^{-2}$ s $^{-1}$ . However this calculation is merely based on assumption of K value which may

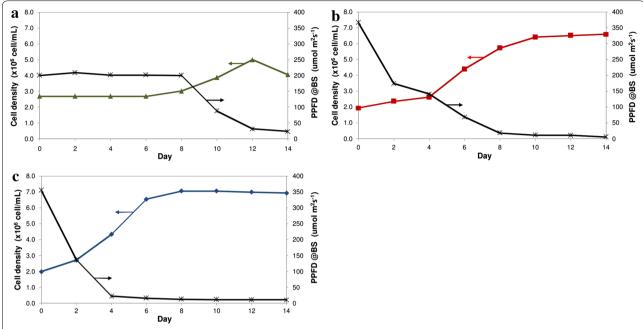


Fig. 4 Relationship of light intensity (PPFD at BS) and cell density (cell growth condition) for each experiment: **a** Experiment 1 at initial 200–220  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and photoperiod 12:12 h, **b** Experiment 2 at initial 350–370  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and photoperiod 12:12 h, and **c** Experiment 3 at initial 350–370  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and photoperiod 18:6 h

vary over the course of batch and depends on the spectrum of light being used. In conclusion, the mean light intensity from the graphs intersection point such as in Fig. 4c was preferably chosen for experiment 3, which is  $140 \, \mu \text{mol m}^{-2} \text{s}^{-1}$  as the mean light intensity, and the efficiency of light utilization,  $Y_{x,E}$  of this PBR was calculated as  $9.0 \times 10^9 \, \text{cell/mol photon}$  (0.49 g/mol photon).

In terms of lipid production, all experiments showed a gradual increase of lipid content as the culture grew. The amount of lipid content was represented by the fluorescence intensity value as shown in Fig. 5. The highest lipid content was achieved in experiment 3 at 91 a.u. followed by experiment 2 and 1 respectively at 78 and 59 a.u. The findings revealed that high cell density reflects high lipid content.

In conclusion, the results showed that batch cultivation in this PBR was feasible, however more work are required to optimize the light parameters and cultivation method to achieve higher cell density. The comparison of previous lab scale work and current work are summarized in Table 1, while the comparison of current PBR and other researchers' findings are compiled in Table 2. It is observed that the results obtained for this scaling up are quite different from the lab scale results. The highest specific growth rate achieved was 0.22 day $^{-1}$  compared to the last result which was 1.60 day $^{-1}$ , while maximum cell density was 7.1  $\times$  10 $^6$  cell/mL compared to the last result which was 10.8  $\times$  10 $^6$  cell/mL. This

difference however can be tolerated since the scale-up factor was at 40 times in terms of cultivation volume. Biomass productivity for this PBR  $(0.04 \text{ g L}^{-1}\text{day}^{-1})$  is at the lower side compared to other reported PBR results  $(0.02-0.85 \text{ g L}^{-1}\text{day}^{-1})$ . The closest comparison is the internally illuminated column (IIC) for Nannochloropsis salina (18 L) using fluorescent lamp at light intensity 91  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (Pegallapati and Nirmalakhandan 2013). In batch culture with 0.035 %  $\mathrm{CO}_2$ -air ratio, the biomass productivity was 0.017 g L<sup>-1</sup>day<sup>-1</sup>, which is lower than this study. However, the biomass productivity was further improved to 0.104 g L<sup>-1</sup>day<sup>-1</sup> by using fed-batch mode and increasing the CO2-air ratio up to 1 %. Mixture of CO<sub>2</sub>-air was also part of strategy to achieve high biomass productivity in 140L IIC (Zitelli et al. 2003), with combined illumination of 400 W metal halide lamp and natural light (outdoor) in continuous mode cultivation. These aspects will be reviewed for our future improvement study.

# Conclusion

The cultivation of *Nannochloropsis* sp. in internally illuminated PBR using combination of blue and red LED, with narrow beam angle was successfully performed. It was found that higher growth rate achieved at higher light intensity 350–370  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and longer photoperiod 18:6. Usage of high intensity LED with narrow beam angle possibly helped in improving microalgae

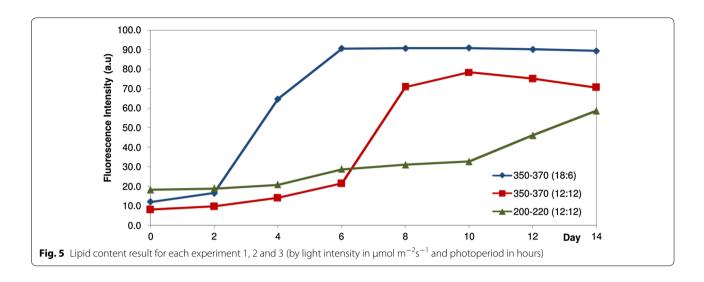


Table 1 Summary of the current work and previous (lab scale) results

PPFD at BS (μmol m <sup>-2</sup> s <sup>-1</sup> )	Photoperiod light:dark (hours)	Spec. growth rate, μ (day <sup>-1</sup> )	Div. rate, <i>k</i> (day <sup>-1</sup> )	Initial cell density (×10 <sup>6</sup> cell/mL)	Max. cell density (×10 <sup>6</sup> cell/mL)	Total volume (L)	Ref.
200–220	12:12	0.126	0.182	2.7	5.0	20	
350-370	12:12	0.195	0.282	1.9	6.4	20	
	18:06	0.220	0.317	2.0	7.1	20	
200 <sup>a</sup>	24:00	1.600	2.308	2.0	10.8	0.5	Teo et al. (2014b)

<sup>&</sup>lt;sup>a</sup> Refer to incident light PPFD

Table 2 Comparison of the current work and other researcher results

PBR type <sup>a</sup>	Micro- algae species <sup>b</sup>	Biomass Productivity (g L <sup>-1</sup> day <sup>-1</sup> )	PPFD (μmol m <sup>-2</sup> s <sup>-1</sup> )	Artificial light	Ref.
This study (IIC)	N	0.02-0.04	350–370	LED	
IIC	NS	0.02-0.10	91	Fluorescent	Pegallapati and Nirmalakhandan (2013)
CY	NO	0.3-0.48	300	Fluorescent	Chiu et al. (2009)
IIC	N	0.17-0.20	89–175	Metal halide	Zittelli et al. (2003)
FP	N	0.61-0.85	230	Fluorescent	Zittelli et al. (2000)

<sup>&</sup>lt;sup>a</sup> Reflected PBR type

IIC, internally illuminated column; CY, cylinder; FP, flat panel; N, Nannochloropsis sp.; NS, Nannochloropsis salina; NO, Nannochloropsis oculata

cultivation in deep culture and high volume such as bubble column PBR, provided that optimum initial cell density, lighting parameter and cultivation method to be reviewed.

# Authors' contributions

MT, CLT, Al, AMY carried out the overall experiments, computational experiments and drafted the manuscript. All authors read and approved the final manuscript.

### Author details

<sup>1</sup> Faculty of Mechanical Engineering, Universiti Teknologi Malaysia, UTM Skudai, 81310 Johor Bahru, Johor, Malaysia. <sup>2</sup> Department of Bioprocess Engineering, Faculty of Chemical & Energy Engineering, c/o Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, UTM Skudai, 81310 Johor Bahru, Johor, Malaysia.

## Acknowledgements

Financial support from Universiti Teknologi Malaysia (Flagship Grant/QJ13000.2409.02G33) and QJ130000.21A2.02E32 for this research is gratefully acknowledged.

<sup>&</sup>lt;sup>b</sup> Reflected Microalgae Species

#### Competing interests

The authors declare that they have no competing interests.

Received: 11 January 2016 Accepted: 29 June 2016 Published online: 07 July 2016

## References

- Alsull M, Omar WMW (2012) Responses of *Tetraselmis* sp. and *Nannochloropsis* sp. isolated from Penang National Park Coastal Waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. In: International conference on chemical, ecology and environmental sciences (ICEES) 12–15
- Atta M, Idris A, Bukhari A, Wahidin S (2013) Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. Bioresour Technol 148:373–378
- Blanken W, Cuaresma M, Wijffels RH, Janssen M (2013) Cultivation of microalgae on artificial light comes at a cost. Algal Res 2:333–340
- Bong SC, Loh SP (2013) A study of fatty acid composition and tocopherol content of lipid extracted from marine microalgae, *Nannochloropsis oculata* and *Tetraselmis suecica*, using solvent extraction and supercritical fluid extraction. Int Food Res J 20(2):721–729
- Chen W, Sommerfeld M, Hu Q (2011) Microwave-assisted Nile red method for in vivo quantification of neutral lipids in microalgae. Bioresour Technol 102:135–141
- Chisti Y (2007) Biodiesel from microalgae. Biotechnol. Adv 25:294-306
- Chiu SY, Kao CY, Ong SC, Chen CH, Lin CS (2009) Lipid accumulation and  ${\rm CO_2}$  utilization of Nannochloropsis oculata in response to  ${\rm CO_2}$  aeration. Bioresour Technol 100:833–838
- Csogor Z, Herrenbauer M, Schmidt K, Posten C (2001) Light distribution in a novel photobioreactor—modelling for optimization. J Appl Phycol 13:325–333
- de Mooij T, de Vries G, Latsos C, Wijffelsa RH, Janssen M (2016) Impact of light color on photobioreactor productivity. Algal Res 15:32–42
- Fu W, Gudmundsson O, Feist AM, Herjolfsson G, Brynjolfsson S, Palsson BØ (2012) Maximizing biomass productivity and cell density of *Chlorella vulgaris* by using light-emitting diode-based photobioreactor. J Biotechnol 161:242–249
- Grima EM, Perez JAS, Camacho FG, Sevilla JMF, Fernandez FGA (1996) Productivity analysis of outdoor chemostat culture in tubular air-lift photobiore-actors. J Appl Phycol 8:369–380
- Hannon M, Gimpell J, Tran M, Rasala B, Mayfield S (2010) Biofuels from algae: challenges and potential. Biofuels 1(5):763–784
- Huang Q, Yao L, Liu T, Yang J (2012) Simulation of the light evolution in an annular photobioreactor for the cultivation of *Porphyridium cruentum*. Chem Eng Sci 84:718–726
- Lee C, Palsson B (1994) High-density algal photobioreactors using light-emitting diodes. Biotechnol Bioeng 44:1161–1167
- Ogbonna JC, Tanaka H (2000) Light requirement and photosynthetic cell cultivation—development of processes for efficient light utilization in photobioreactors. J Appl Phycol 12:207–218
- Ogbonna JC, Yada H, Masui H, Tanaka H (1996) A novel internally illuminated stirred tank photobioreactor large-scale cultivation of photosynthetic cells. J Ferment Bioeng 82:61–67
- Olivieri G, Salatino P, Marzocchella A (2014) Advances in photobioreactors for intensive microalgal production: configurations, operating strategies and applications. J Chem Technol Biotechnol 89:178–195
- Pegallapati AK, Nirmalakhandan N (2013) Internally illuminated photobioreactor for algal cultivation under carbon dioxide-supplementation: performance evaluation. Renew Energy 56:129–135

- Pegallapati AK, Arudchelvam Y, Nirmalakhandan N (2012) Energy-efficient photobioreactor configuration for algal biomass production. Bioresour Technol 126:266–273
- Posten C (2009) Review: design principles of photo-bioreactors for cultivation of microalgae. Eng Life Sci 3:165–177
- Quinn JC, Yates T, Douglas N, Weyer K, Butler J, Bradley TH, Lammers PJ (2012)

  Nannochloropsis production metrics in a scalable outdoor photobioreactor for commercial applications. Bioresour Technol 117:164–171
- Ra C-H, Kang C-H, Jung J-H, Jeong G-T, Kim S-K (2016) Effect of light-emitting diodes (LEDs) on the accumulation of lipid content using two-phase culture process with three microalgae. Bioresour Technol 212:254–261
- Rawat I, Ranjith Kumar R, Mutanda T, Bux F (2013) Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl Energy 103:444–467
- Richmond A (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Blackwell Publishing, Hoboken, pp. 20–39
- Rocha JMS, Garcia JEC, Henriques MHF (2003) Growth aspects of the marine microalga *Nannochloropsis gaditana*. Biomol Eng 20(4–6):237–242
- Silva Benavides AM, Torzillo G, Kopecký J, Masojídek J (2013) Productivity and biochemical composition of *Phaeodactylum tricornutum (Bacillariophyceae)* cultures grown outdoors in tubular photobioreactors and open ponds. Biomass Bioenergy 54:115–122
- Suh IS, Lee SB (2001) Cultivation of a cyanobacterium in an internally radiating air-lift photobioreactor. J Appl Phycol 13:381–388
- Teo CL, Atta M, Bukhari A, Taisir M, Yusuf AM, Idris A (2014a) Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. Bioresour Technol 162:38–44
- Teo CL, Idris A, Zain NAM, Taisir M (2014b) Synergistic effect of optimizing light-emitting diode illumination quality and intensity to manipulate composition of fatty acid methyl esters from *Nannochloropsis* sp. Bioresour Technol 173:284–290
- Wahidin S, Idris A, Muhamad Shaleh ST (2013) The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nan-nochloropsis* sp. Bioresour Technol 129:7–11
- Xue S, Zhang Q, Wu X, Yan C, Cong W (2013) A novel photobioreactor structure using optical fibers as inner light source to fulfill flashing light effects of microalgae. Bioresour Technol 138:141–147
- Yan C, Munoz R, Zhu L, Wang Y (2016) The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae *Chlorella* sp. Energy 106:554–561
- Zijffers JWF, Schippers KJ, Zheng K, Janssen M, Tramper J, Wijffels RH (2010) Maximum photosynthetic yield of green microalgae in photobioreactors. Mar Biotechnol 12:708–718
- Zittelli GC, Pastorelli R, Tredici MR (2000) A modular flat panel photobioreactor (MFPP) for indoor mass cultivation of *Nannochloropsis* sp. under artificial illumination. J Appl Phycol 12:521–526
- Zittelli GC, Rodolfi L, Tredici MR (2003) Mass cultivation of *Nannochloropsis* sp. in annular reactors. J Appl Phycol 15:107–114

# Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Immediate publication on acceptance
- ► Open access: articles freely available online
- ► High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ▶ springeropen.com