Evaluation of a modified photobioreactor performance for bio-fixation of carbon dioxide by *Chlorella vulgaris* at different light intensities

Gita Naderi, Moses O. Tade, Hussein Znad*
Department of Chemical Engineering, Curtin University, GPO Box U1987 Perth WA 6845, Australia
*Correspondence: Dr. Hussein Znad (E-mail: h.znad@curtin.edu.au), Department of Chemical Engineering, Curtin University, GPO Box U1987 Perth WA 6845, Australia

ABSTRACT

The performance of a modified bioreactor inside a light enclosure for carbon dioxide (CO_2) bio-fixation by *Chlorella vulgaris* was investigated. The influence of different light intensities on CO_2 bio-fixation and biomass production rates was evaluated. The results showed that the photon flux available to microalgal cultures can be a key issue in properly optimizing microalgae photobioreactor performance, particularly at high cell concentrations. Although the optimal pH values for *C. vulgaris* range from 6-8, cell growth can take place even at pH 4 and 10. Batch microalgal cultivation in a photobioreactor was used to investigate the effect of different light intensities, including 30, 50, 100, 185 and 300 µmol m^{-2} s^{-1}. The maximum biomass concentration of 1.83 g L^{-1} was obtained at a light intensity of 100 µmol m^{-2} s^{-1} and 2 L min^{-1} of 2% CO_2 enriched air aeration.
Keywords: photobioreactor; *Chlorella vulgaris*; CO_2 biofixation; light intensity

1. Introduction

Currently, the world is facing crises of environmental degradation and worldwide health due to elevation of greenhouse gases. It is believed that carbon dioxide (CO_2) accounts for up to 68% of the total greenhouse gases that are responsible for global climate change [1]. Both environmental impact and limited reserves of fossil fuel have motivated many researchers to investigate commercialization of microalgae culturing for both CO_2 biofixation and biofuel production.

Some research has been successful in reducing CO_2 emissions, but we are still seeking more effective strategies for CO_2 sequestration. CO_2 mitigation strategies can be classified into three groups: (1) chemical reaction-based approaches for instant carbonation/de-carbonation reactions [2]; (2) direct injection to the underground or to the ocean, called carbon capture and storage (CCS); and (3) biological CO_2 mitigation, which is the biologic conversion of CO_2 into organic matter. Because chemical reaction-based and direct injection strategies are relatively costly and energy-consuming, biological CO_2 fixation has received much attention as an alternative strategy. Moreover, CO_2 biofixation leads to biomass production which can be used as a source of energy and feedstock for biofuel production as well as other valuable products [2]. Biological CO_2 mitigation can be achieved

Received: December 23, 2014; revised: April 07, 2015; accepted: May 20, 2015

To be cited as: Chem. Eng. Technol. 10.1002/ceat.201400790

Link to final VoR: http://dx.doi.org/10.1002/ceat.201400790

through both terrestrial plants and an enormous number of photosynthetic microorganisms. However, while the potential CO_2 absorption by terrestrial plants is only 3-6% of the CO_2 discharge from fossil fuels, microalgae have the ability to absorb CO_2 with the efficiency of 10 to 50 times that amount [1, 2]. CO_2 biofixation through microalgae culturing is one of the most promising ways to mitigate CO_2 because of its sustainability and eco-friendliness, as well as the possibilities of also producing high valued co-products such as biofuel and supplementary foods or combining this technique with wastewater treatment.

Many factors such as temperature, pH, light intensity, aeration rate and agitation affect microalgae growth. However, light intensity is one of the most significant factors because if it is insufficient or exceedingly high, it can limit or inhibit microalgae growth. Furthermore, the light/dark cycle is another critical factor in the growth of microalgae [3]. At moderate light intensities in the batch cultivation of *C. vulgaris*, *Chlorella pyrenoidosa*, and *Aphanothece microscopic Nageli*, the growth rate increased as the light duration increased [4, 5].

Nevertheless, due to shading effects, including photon-absorption by cells and scattering by particles, there is a light gradient inside the culture, especially in dense cultures. Many researchers have investigated light illumination factors and the effect of light intensity to optimize microalgae growth or CO_2 mitigation. Recently Wahidin et al. [6] cultivated *Nannochloropsis* in a photobioreactor with three different light intensities of 50, 100 and 200 µmol m^{-2} s^{-1} and reported a maximum cell concentration of 6.5 x 10^7 cells ml^{-1} under 100 µmol m^{-2} s^{-1} and an 18 hour day-photoperiod. Martinez et al. [7] reported 2.07 gCO_2 L^{-1} d^{-1} optimum biofixation rate when culturing *Synechocystis* sp. at a light intensity of 686 µmol m^{-2} s^{-1}. An average CO_2 mitigation of 166 mg d^{-1} was achieved when *Spirulina platensis* was cultivated at 90 µmol m^{-2} s^{-1} light intensity, and then the daily average of CO_2 consumption improved to 229 mg d^{-1}, due to the increase in light intensity to 125 µmol m^{-2} s^{-1} after 37 days [8]. In addition to the interaction of other factors such as temperature or photoperiod on optimum light intensity, different strains also vary in their optimum conditions for maximum biomass production or CO_2 removal. *Chlorella vulgaris* is one of the promising microalgae species suitable for CO_2 sequestration due to fast growing, relatively high carbon content and also tolerance to high temperature and CO_2 concentration [9, 10]. Bhola et al. [11] stated that *C. vulgaris* can tolerate light intensity between 150 to 350 µmol m^{-2} s^{-1} and could achieve a 6.17 mg L^{-1} h^{-1} CO_2 fixation rate. Tab. 1 shows clearly that there is no optimum light intensity for particular microalgae strains as there are many factors could affect.

Table 1

In this study, the biomass production rate and CO_2 biofixation rate by *C. vulgaris* in a batch photobioreactor at different light intensities were investigated. It has been shown that this species has high productivity and CO_2 fixation ability [26]. A light enclosure was designed for better and controlled illumination for the available bioreactor to explore the optimum light intensity for *C. vulgaris*. Operational conditions of the photobioreactor such as pH, dissolved oxygen (DO) and inlet CO_2 concentration were measured and discussed.

2. Material and Methods

2.1. Microalgae, medium and cultivation conditions

The fresh water microalgae strain *Chlorella vulgaris* was cultivated in this work. A culture of *C. vulgaris* (CCAP 211/11B, CS-42) was obtained from CSIRO, dispatched in 250 ml tissue-culture flask and immediately subcultured to fresh medium after arrival.

C. vulgaris was cultured in fresh MLA medium based on the CSIRO recipe, which has been derived from ASM-1 medium reported in Gorhan et al. [27]. This medium contains the following compositions (in mg L^{-1}): $MgSO_4.7H_2O$ 49.4; $NaNO_3$ 170; K_2HPO_4 34.8; H_3BO_3 2.47; H_2SeO_3 1.29x10^{-3};

Biotin $50x10^{-6}$; Vitamin B_{12} $50x10^{-6}$; Thiamine HCl 0.1; Na_2EDTA 4.36; $FeCl_3.6H_2O$ 1.58; $MnCl_2.4H_2O$ 0.36; $CuSO_4.5H_2O$ 0.01; $ZnSO_4.7H_2O$ 0.022; $CoCl_2.6H_2O$ 0.01; $Na_2MoO_4.2H_2O$ $6x10^{-3}$; $NaHCO_3$ 17.5; $CaCl_2.2H_2O$ 29.4.

Stock cultures were propagated every two weeks by aseptically transferring 20 ml of old culture to 200 ml autoclaved fresh medium in a 250-ml Erlenmeyer flask. All stock cultures including grandparent, parent and daughter were kept inside the refrigerated incubator shaker equipped with four 10 W daylight fluorescent tubes, so that a photon flux density of approximately 80 µmol m^{-2} s^{-1} was supplied to the flasks, with a light/dark photoperiod of 12/12 hours. Temperature was controlled at 20 ^{0}C. To prevent sedimentation, Erlenmeyer flasks were continuously shaken in a rotational shaker at 200 rpm and occasionally shaken adequately by hand to prevent microalgae from adhering to the inside surface of the glass.

The inoculation was performed by aseptically transferring stock culture to autoclaved fresh medium with an inoculation ratio of 10:100 to maintain an initial concentration of 0.01 g L^{-1}.

Culturing was carried out at 20 ^{0}C by bubbling 2% CO_2-enriched air at a flow rate of 2 L min^{-1} for all the experiments, by adjusting the individual flow rates of air and CO_2 lines to 2 and 1 L min^{-1} respectively. The impeller was set at 200 rpm for better mixing and prevention of sedimentation. Samples were withdrawn in 24-hour intervals at the same time for further analysis.

2.2. Photobioreactor

A three-litre bench-top bioreactor (New Brunswick's BioFlo®/CelliGen® 115) was used in this work. It consists of a cylindrical glass vessel with a two litre working volume surrounded by a water jacket. Precise temperature control was achieved through cool a water jacket, heater and PI controller. The experiment was performed in batch mode so that gas was continuously aerated to the batch broth inside the bioreactor.
DO and pH were monitored daily through the DO and pH meters installed at the top of the bioreactor. Culture temperature was measure via a RTD sensor fixed in the thermo-well port and controlled by a PI controller.

With separate gas cylinders of atmospheric air and CO_2, two rotameters and a CO_2 meter (G110-10N, VIASENSOR), it was easy to supply gas with any desired concentration of CO_2. The inlet gas mixture is first passed through a 0.2 µm PTFE membrane (Acro 50 PTFE vent filter, PALL) to remove living organisms and then sparged into the bottom of the bioreactor. A schematic diagram of the photobioreactor system are shown in Fig. 1.

Fig. 1

Five experiments were conducted at light intensities of 30, 50, 100, 185 and 300 µmol m^{-2} s^{-1} to investigate the effect of light intensity. A light enclosure was designed and built in the lab which was an octagonal prism and sixteen 6 W cool white fluorescent tubes were affixed inside. To prevent high temperatures inside the light enclosure, two fans were affixed on the top of the light enclosure. Both the number of the lamps and distance between the lamps and surface of the bioreactor are adjustable, so they can easily furnish various light intensities emitted to the bioreactor. The bioreactor was surrounded with the light enclosure and it was adjusted so that a light intensity of 30, 50, 100, 185 and 300 µmol m^{-2} s^{-1} illuminated the bioreactor surface. For instance, a light intensity of 50 µmol m^{-2} s^{-1} was furnished by eight 6 W fluorescent lamps located at approximately 10 cm from bioreactor surface.

Table 2 summarizes the light enclosure set-up.

Table 2

2.3. Analytical procedures

The light intensity on the surface of the bioreactor was measured using a quantum sensor (LI-192SA, LI-Core Inc.) connected to a light meter (LI-250, LI-COR Inc.). After measuring the light intensity at 25 distinct locations on the surface of the vessel, average incident light intensity was calculated. These 25 locations were taken at five vertical positions and five evenly distributed points around the half perimeter of the vessel due to symmetry of the vessel and illumination. Then, the average light intensity was calculated by taking into account the weighted average of the measurements.

Optical density (OD) of samples was measured using a spectrophotometer (UV-Vis SP8001). By measuring the absorption spectrum over 400-700 nm wavelength, or Photosynthetically Active Radiation (PAR), the absorption peaks for *C. vulgaris* were specified. It was observed that the maximum absorbance is approximately 440 nm and 684 nm. As it has been investigated by Fan et al. [28], absorption peaks for this strain are located in blue (460-470 nm) and red (640-690) light bands. On the other hand, it has been shown that red light wavelengths are more responsible for photosynthesis, and therefore significantly higher growth is achieved in this wavelength range in comparison with 590-600 nm for yellow light or 460-470 nm for blue light [29]. Therefore, OD of samples has been measured at 684 nm.

Biomass concentration was measured using the dry weight method. A known volume of the biomass samples were filtered through pre-weighted, pre-combusted Whatman glass fibre filters (GF/C, 1.2 µm), then rinsed by 10 ml distilled water and dried in an oven overnight at 105 ^{0}C for 24 hours and reweighed to determine the dry weight of the filtered microalgae.

Triplicate measurements were carried out for both cell dry weight and OD at 684 nm for nine samples of different known concentrations with OD between 0.1 - 0.9. Then, the following linear calibration equation with a correlation coefficient of 0.9968 was obtained from plotting dry cell weight (DCW) over optical density at 684 (OD_{684}):

$$DCW = 0.2036\ \ OD_{684} - 0.0006 \quad (R^2 = 0.9968) \tag{1}$$

It should be noted that the calibration curve is varied for different species. This equation is very close to the calibration equation obtained by Sacasa [30], equation (2), which measured OD for the same strain at 686 nm.

$$DCW = 0.2936 OD_{686} + 0.0007 \tag{2}$$

Elemental composition of the *C. vulgaris* and total carbon content was determined using an element analyser (2400 Series II CHNS/O).

2.4. Microalgae growth, biomass productivity, CO_2 biofixation

Microalgae growth was monitored by measuring OD and then DCW was calculated using equation (1). The specific growth rate was calculated according to the equation:

$$\mu = \frac{Ln(X_2 / X_1)}{(t_2 - t_1)} \tag{3}$$

Where X_2 and X_1 are the biomass concentration (g L^{-1}) at the time t_2 and t_1 (d), respectively.

Biomass productivity (g DW L^{-1} d^{-1}) was calculated by the following equation:

$$Biomass\ productivity,\ P = \frac{X_t - X_0}{t} \quad (4)$$

Where X_t is the final cell concentration (g L^{-1}) in cultivation time t and X_0 is the initial cell concentration (g L^{-1}).

The total carbon content of dried cell (% C) was determined by the elemental analyser. Thus, the CO_2 biofixation rate (g CO_2 L^{-1} d^{-1}) can be calculated according to equation (5).

$$CO_2\ biofixation\ rate = \%C \times P \times \frac{M_{CO_2}}{M_C} \quad (5)$$

Where M_{CO_2} and M_C are the molecular weights of CO_2 and carbon, respectively.

3. Results and Discussion

3.1 Effect of light intensity on C. vulgaris growth

Fig. 2 illustrates the growth curve of the fresh water *C. vulgaris* at five different light intensities, 30, 50, 100, 185 and 300 µmol m^{-2} s^{-1}; the light intensities were maintained for 17 days. The culture showed an exponential growth phase after a two days lag phase up to six to seven days. The maximum cell concentration of 1.25 g L^{-1} in exponential growth was achieved at 100 µmol m^{-2} s^{-1} light intensity in comparison with 0.33, 0.98, 0.60 and 0.69 g L^{-1} at 30, 50, 185 and 300 µmol m^{-2} s^{-1} light intensities, respectively. Then, although a slowdown in cell growth was observed, it did not stop completely. Such diauxic nature mainly due to adding 20 ml fresh medium during the period between 6 to 8 days in all experiments, to compensate the withdrawn samples, such addition resulted in diluting the cultures and low biomass measured during that period, Fig. 2. After the 8th cultivation day, the cell biomass continue to increase but with less growth rate until approaching the stationary phase (after 14 cultivation days). The slow growth rate could be attributed to the shading effect associated with less available photon flux in the dens culture. Moreover, light scattering causes less available photon flux for the deep part of the culture as well as a sharp decrease in light gradient to form in the cross-section of the vessel.

It's well-known that the light intensity dramatically decreases as the light travels into the culture, due to absorption by the light-harvesting pigments of algae and scattering by algal cells [31]. Despite its inaccuracy in dense algal cultures, one of the widely used model for calculating the light absorption and penetration depth is Beer-Lambert's law [32, 33], more accurate model was proposed by Kim et al [31].

Fig. 2

A maximum biomass of 1.83 g L^{-1} was obtained on the last day with a light intensity of 100 µmol m^{-2} s^{-1}. A rise in light intensity from 100 to 185 and 300 µmol m^{-2} s^{-1} led to drop in biomass concentration from 1.83 to 1.34 g L^{-1}. Even cultivation at 50 µmol m^{-2} s^{1} photon flux presented better growth and higher cell concentration than the very high light intensities; thus, 1.58 g L^{-1} biomass was obtained when the bioreactor was illuminated under 50 µmol m^{-2} s^{-1} while the maximum biomass density for light intensities of 185 and 300 µmol m^{-2} s^{-1} was only 1.34 g L^{-1}. Considering this result and the idea that cell growth is almost the same at light intensities of 185 and 300 µmol m^{-2} s^{-1}, it can be concluded that the biomass growth is inhibited at 185 and 300 µmol m^{-2} s^{-1}. In the phenomena of photoinhibition that occurs at high light intensities, the photosynthetic receptor system of the cells is damaged and consequently biomass growth decreases. Additionally, the colour of the cells changes and they appear lighter or even brown in the case of very high light intensities [6].

On the other hand, under the photolimitation condition, microalgae growth is limited because there is not sufficient light. It was observed that microalgae did not grow well when 30 µmol m^{-2} s^{-1} of light intensity was exposed to the bioreactor, and the biomass reached to 0.33 g L^{-1} in six days. With an increase in light intensity from 30 to 50 µmol m^{-2} s^{-1}, biomass density increased to 0.98 g L^{-1}, almost three times the density for the same cultivation time.

Because light provides the required energy for photosynthesis, it is one of the significant factors affecting microalgae growth along with other factors such as pH, temperature and aeration rate. However, both phenomena of photolimitation and photoinhibition, as well as the change in the light gradient inside the vessel with cultivation time and cell density, make light regime analysis more complicated.

3.2. Effect of biomass growth on pH and DO

The pH value of the MLA medium is approximately 7.8; however, after autoclaving it increases and again dramatically drops when sparging CO_2-enriched air. The pH of the autoclaved medium rose to nearly 8.1 due to high temperature and degassing in the autoclave, and after gas injection it sharply dropped to approximately 5.7 (at 30 µmol m^{-2} s^{-1} light intensity) as a result of increasing dissolved CO_2. After inoculation, the pH level started to slightly increase when cells started to grow because inorganic carbon is consumed by the microalgae, Fig. 3; however, a few days later, the pH value almost levelled off. Generally, pH values ranged from 5.7 to 7.2 during cultivation in these experiments, which do not inhibit *C. vulgaris* growth [34]; even at the highest level of pH up to 7.2, no negative effect on growth was observed.

Fig. 3

A sharper exponential growth rate in the first six days accompanied by a sharper increase in pH was observed, and after a reduction in growth in the following days, less change in pH occurred. As it can be observed after six days of cultivation, the pH in each experiment became nearly constant and the final pH values (after 11 cultivation days) in the different experiments ranged between 6.4 and 7.2.

Two factors can affect the final pH values: the percentage of the carbon dioxide injected into the bioreactor and the amount of the biomass produced. High inlet CO_2 and less biomass production results in a lower pH value. In all experiments, CO_2 percentage in the inlet gas was adjusted to be 2%. The minimum pH of 6.4 was observed at a light intensity 30 µmol m^{-2} s^{-1} due to less cell growth. In contrast, the maximum pH of 7.2 was achieved at a light intensity 100 µmol m^{-2} s^{-1} which was associated with more biomass production (Fig. 3).

The same pH evolution trend has been reported by Concas et al. [35] when they cultured *C. vulgaris* in a semi-batch photobioreactor. The pH of the medium (7.5) decreased to 5.6 after gas inlet was added and then slowly increased to 6.2. In addition, a slight increase in pH from 6.3 to 7 followed by little fluctuation in pH has been reported by Li et al. [36] when *C. vulgaris* was cultured in 0.035 L min^{-1} aeration with 15% CO_2 enriched air. However, steep variation in pH from 6.5 to 8.3 has been observed when the aeration was reduced to 0.025 L min^{-1}, which can cause a negative effect on alga growth. As discussed by Lutzu et al. [34], that the optimal pH value for *C. vulgaris* ranges from 6-8, cell growth can take place even at pH 4 and 10. Therefore, this strain can be a good choice for direct CO_2 sequestration from flue gas.

CO_2 as a source of carbon was consumed and converted to biomass via photosynthesis. On the other hand, aeration of CO_2 to the medium has a reduction effect on pH due to the reaction of CO_2 with water forming carbonic acid [37]. However, cells grew and subsequently pH increased because of CO_2 consumption by photosynthesis reaction [11]. Meanwhile, there was a sharp increase in pH in the first few days (exponential growth phase) and then a slight increase of pH after reaching the stationary growth phase; which can be seen in Fig. 4 for experiment at 100 µmol m^{-2} s^{-1}. This shows

that there is a close relationship between pH and cell production. Consequently, photosynthetic growth and CO_2 utilization rate will be limited in very high or very low pH values. Therefore, investigating pH evolution in microalga biomass culturing can be a key issue to properly optimize a microalgae photobioreactor. In particular, this aspect is significant when highly concentrated CO_2 flue gases from power plants as the source of CO_2 are used. In this case, the pH level can reach very low values and may inhibit microalgae growth [9].

Fig. 4

Fig. 5 illustrates the variation in both cell concentration at days 6 and 17 and final pH value with light intensity. It was observed that the maximum pH value (7.22), which is accompanied by the highest biomass concentration, occurred at 100 µmol m^{-2} s^{-1} of light intensity. Meanwhile, the second maximum pH (7.14), which was obtained at 50 µmol m^{-2} s^{-1} photon flux, was associated with the second maximum cell concentration. The lowest pH (6.4) occurred in the experiment with minimum biomass production and a light intensity of 30 µmol m^{-2} s^{-1}.

Fig. 5

Fig. 6 shows the biomass growth curve and dissolved oxygen over cultivation time for three experiments with 50, 100 and 185 µmol m^{-2} s^{-1} light intensities. Generally, there was a noticeable rise the first few days and then a reduction in DO observed in all experiments. Generally the DO increases with biomass and CO_2 fixation. In this study the range of DO% variation was very narrow between 95% and 105% which was saturated and not accurate enough and to show the expected trend, however, overall it's very clear from Fig. 6 that there is an increase in DO% from day 0 until day 12 where the stationary phase started, Fig.6.

Indeed, exponential cell growth caused an increase in DO levels and then a decrease in biomass growth rate accompanied by less oxygen and a decrease in DO. These results suggest that the steep change in DO during the exponential growth phase of microalgae is due to the photosynthesis, CO_2 consumption and O_2 generation. Consequently, DO monitoring can be used to predict the health of the microalgae. Chai et al. [19] observed a gradual increase in the daily DO peak during the exponential growth phase and then after, reaching 135%. It decreased during stationary phase, for a batch culture of *Chlorococcum* sp. was investigated.

Fig. 6

3.3. Specific growth rate, productivity and CO_2 biofixation rate evolution

The growth of the cells in the present study is being measured and the specific growth rate is being calculated by equation (3). Biomass productivity and CO_2 biofixation rate are calculated using equations (4) and (5). Fig. 7 illustrates specific growth rate, biomass production rate and CO_2 biofixation rate at different light intensities. Specific growth rate increased with an increase in light intensity. Increasing the light intensity from 50 to 100 µmol m^{-2} s^{-1} resulted in enhancing the specific growth rate from 0.52 to 0.8 d^{-1}. Specific growth rate increased with increasing light intensity and no sharp change occurred. It finally reached a maximum of 0.93 d^{-1} at a light intensity of 300 µmol m^{-2} s^{-1}.

Fig. 7

The specific growth rates in these experiments were calculated as 0.455, 0.522, 0.799, 0.878 and 0.933 d^{-1} at 30, 50, 100, 185 and 300 light intensities, respectively. Maximum growth rates of 0.04 and 0.06 h^{-1} equated to 0.96 and 1.44 d^{-1}, and this has been reported for the same strain by Jacob et al. [38] and Lutzu et al. [34] when 15% and 100% CO_2 were injected. Sacasa et al. [30] cultivated the same strain in a similar bioreactor with 2 L min^{-1} of 3% CO_2 inlet gas and achieved a maximum growth rate of 0.172 d^{-1} and a maximum cell concentration of 0.470 g L^{-1}.

Despite the continuous increase of the specific growth rate with an increase of light intensity, the production rate did not show the same trend. Productivity increased when the light intensity changed from 30 to 50 and then 100 µmol m^{-2} s^{-1}; nevertheless, it was reduced at light intensities of 185 and 300 µmol m^{-2} s^{-1}. Productivity reached 0.21 g DW $L^{-1}d^{-1}$ at a light intensity of 100 µmol m^{-2} s^{-1} which was maximized in comparison with lower and higher light intensities. Similarly, CO_2 biofixation showed a similar trend because it is proportional to the production rate. Maximum CO_2 biofixation achieved at 100 µmol m^{-2} s^{-1} was 0.45 g CO_2 L^{-1} d^{-1}. This result was better than those obtained by Yun et al. [16], who cultivated *C. vulgaris* at 27 ^{0}C, 15% CO_2 and 110 µmol m^{-2} s^{-1} and obtained a rate of 0.312 g L^{-1} d^{-1}, and by Sydney et al. [24], who achieved 0.252 g CO_2 L^{-1} d^{-1} for cultivation of the same strain at 30 ^{0}C, 5% CO_2 and 3500 lux. Ho et al. [39] saw the same trend for production rate and CO_2 biofixation rate when they cultured *Scenedesmus obliquus*. For this strain, productivity is maximized at light intensity of 420 µmol m^{-2} s^{-1} and then decreased with higher light intensity. A rate of 0.128 g CO_2 L^{-1} h^{-1} was removed by *C. vulgaris* when cultivated in a bubble column at 25 ^{0}C, 1.25 L min^{-1} gas flow rate and 10800 lx light intensity [28]. Furthermore, daily CO_2 biofixation of 1.96 g d^{-1} was obtained at 1600 µmol m^{-2} s^{-1} for *Synechocystis* Sp. after six days cultivation, which was maximum in comparison with cultivation at lower or higher light intensities [39].

Lower biomass production and CO_2 removal at lower light intensities is due to light limitation, a drop in productivity and CO_2 fixation at higher light intensities occurred because of photoinhibition [13, 6]. Furthermore, in dense cultures, both photolimitation and photoinhibition can take place simultaneously. Increasing light intensity to avoid light limitation in the deep parts of dense cultures may lead to inhibition of microalgae growth at the surface of the bioreactor [39].

Despite a higher specific growth rate in light intensities of 185 and 300 µmol m^{-2} s^{-1} compared with a light intensity of 100 µmol m^{-2} s^{-1}, the production rate was less . This can be considered a result of inhibition. In reality, due to more photon flux available to microalga, it grew faster at first but afterwards, high light intensity damaged the cells and consequently, they did not continue to grow well. Many researchers concentrated on photoinhibition phenomena for variety of species. For instance, Wahidin et al. [6] investigated the influence of light intensity on *Nannochloropsis* sp. and discussed that light intensity above saturation led to an inhibition effect. Microalgae growth can take place in light limitation, light saturation or light inhibition conditions [39]. Saturation light intensity varies for different species and it ranges from 140 to 210 µmol m^{-2} s^{-1} [36]. For example, according to Hanagata et al. [40], 200 µmol m^{-2} s^{-1} is the saturation light intensity for *Chlorella* Sp. and *Scendesmus*.

In this study, a maximum of 0.45 g CO_2 L^{-1} d^{-1} was fixed at a light intensity of 100 µmol m^{-2} s^{-1}. In a study performed by Ryu et al. [25], maximum productivity and maximum biofixation rate of 0.335 g DW L^{-1} d^{-1} and 0.35 g CO_2 d^{-1} have been reported when *Chlorella sp.* was cultivated in 600 ml vertical photobioreactor aerated by 5% CO_2 enriched air. However, they achieved 0.295 g DW L^{-1} d^{-1} productivity and 0.31 g CO_2 d^{-1} biofixation when 2% CO_2 was injected.

4. Conclusions

Batch cultivations were conducted to investigate the effect of light intensity on CO_2 biofixation and biomass productivity. The results revealed that the *C. vulgaris* sp. can grow at a much higher rate at a light intensity of 100 µmol m^{-2} s^{-1} and 2 L min^{-1} aeration rate of 2% CO_2 enriched air. *C. vulgaris* reached a maximum of 1.83 g L^{-1} cell concentration at a 100 µmol m^{-2} s^{-1} light intensity in 17 days while only 1.26, 1.58, 1.34, 1.34 g L^{-1} were achieved at light intensities of 30, 50, 185 and 300, respectively. Low biomass growth in the experiment with a 30 µmol m^{-2} s^{-1} light intensity is due to photolimitation, and on the other hand, 185 and 300 µmol m^{-2} s^{-1} light intensities caused damage to cells and photoinhibition occurred. The results indicate improvement in both maximum growth rate and biomass concentration in light intensity of 100 µmol m^{-2} s^{-1} and 2 L min^{-1} aeration rate which

accompanies higher CO_2 biofixation. Both high growth rate and high carbon content of this strain make it a prospective microalgae species in CO_2 biofixation technology.

Symbols used

DCW	[g dry cell L^{-1}]	Dry cell weight per broth volume
DO	[%]	Dissolved oxygen
M_C	[g mol^{-1}]	Molecular weight of carbon
M_{CO2}	[g mol^{-1}]	Molecular weight of carbon dioxide
P	[g DCW L^{-1} d^{-1}]	Productivity
t	[d]	Cultivation time
X	[g L^{-1}]	Cell concentration
X_0	[g L^{-1}]	Initial cell concentration
Greek symbols		
μ_{max}	[d^{-1}]	Max specific growth rate

References

[1] S. H. Ho, C. Y. Chen, D. J. Lee, J. S. Chang, *Biotechnol. Adv.*. **2011,** 29, 189-198.
[2] B. Wang, Y. Li, N. Wu, C. Q. Lan, *Appl. Microb. Biotechnol.* **2008,** 79, 707-718.
[3] S.-K. Wang, A. R. Stiles, C. Guo, C.-Z. Liu, *Eng. Life Sci.* **2014**, *14*, 550–559.
[4] M. Ponraj, M. F. M. Din, *J. Sci. Ind. Res.* **2013**, *72*, 703–706.
[5] E. Jacob-Lopes, C.H.G. Scoparo, L. M. C. F. Lacerda, T. T. Franco, *Chem. Eng. Process* **2009**, *48*, 306–310.
[6] S. Wahidin, A. Idris, S. R.M. Shaleh, *Bioresour. Technol.* **2013**, 129, 7-11.
[7] L. Martinez, V. Redondas, A. I. Garcia, A. Moran, *J. Chem. Technol. Biotechnol.* **2011**, 86, 681-690.
[8] S. Arata, C. Strazza, A. Lodi, A. Del Borghi, *Chem. Eng. Technol.* **2013**, 36, 91-97.
[9] J. C. M. Pires, M. C.M. Alvim-Ferraz, F. G. Martins, M. Simoes, *Ren. Sustain. Energ. Rev.* **2012**, 16, 3043-3053.
[10] F. M. Salih, *J. of Environ. Protec.* **2011**, *2*, 648-654.
[11] V. Bhola, R. Desikan, S. K. Santosh, K. Subburamu, E. Sanniyasi, F. Bux, *J. Bioscien. Bioeng.* **2011,** 111, 377-382.
[12] C. Lopez, et al., *Bioresour. Technol*., **2009**. 100(23), 5904-5910.
[13] C.L. Chiang, C.M. Lee, and P.C. Chen, *Bioresour. Technol.* **2011,** 102(9), 5400-5405.
[14] J. F. Sánchez, C. V. González-López, F. G. Acién Fernández, J. M. Fernandez Sevilla, E. Molina Grima, *Appl. Microb. and Biotechnol.* **2012**. 94(3), 613-624.
[15] K. Zhang, S. Miyachi, and N. Kurano, *Appl. Microb. Biotechnol.* **2001,** 55(4), 428-433.
[16] Y. S. Yun, S. B. Lee, J. M. Park, C. Lee, J-W. Yang, *J. Chem. Technol. Biotechnol.* **1997**, 69(4), 451-455.
[17] I. Douskova, J. Doucha, K. Livansky, J. Machat, P. Novak, D. Umsova, V. Zachleder, M. Vitova, *Appl. Microb. Biotechnol.* **2009**. 82(1), 179-185.
[18] L. Cheng, L. Zhang, H. Chen, C. Gao, *Separ. Purif. Technol.* **2006,** 50(3), 324-329.
[19] X. Chai, X. Zhao, and W. Baoying, *African J. Biotechnol.* **2012,** 11(29), 7445-7453.
[20] W. Kim, J. Park, G. Gim, S.H. Jeong, C. Kang, D.J. Kim, and S. Kim, *Bioproc. Biosys. Eng.* **2012,** 35(1-2),19-27.

[21] L. Martinez, A. Moran, and A.I. Garcia, *J. Appl. Phycol.* **2012**. 24(1), 125-134
[22] S.-Y. Chiu, C-Y. Kao, C-H. Chen, T-C. Kuan, S-C. Ong, C-S. Lin, *Bioresour. Technol.* **2008**, 99(9), 3389-3396.
[23] M. G. Morais, and J.A.V. Costa, *J. Biotechnol.* **2007**, 129(3): p. 439-445.
[24] E. B. Sydney, W. Sturm, J. C. De Carvalho, V. Thomaz-Soccol, C. Larroche, A. Pandey, C. R. Soccol, . *Bioresour. Technol.* **2010**, 101, 5892-5896.
[25] H. J. Ryu, K. K. Oh, Y. S. Kim, *J. Ind. Eng. Chem.* **2009**, 15, 471-475.
[26] M. Anjos, B. D. Fernandes, A. A. Vicente, J. A. Teixeira, G. Dragone, *Bioresour. Technol.* **2013**, 139, 149-154.
[27] P. R. Gorhan, R. W. McLachlan, U. T. Hammer, *Breb. Int. Ver. Theor. Angew. Limnol. Verh.,* **1964***,19, 796–804.*
[28] L. H. Fan, Y. T. Zhang, L. H. Cheng, L. Zhang, D. S. Tang, H. L. Chen, *Chem. Eng. Technol.* **2007**, 30, 1094-1099
[29] Z. Ge, H. Zhang, Y. Zhang, C. Yan, Y., Zhao, *J. Environ. Heal. Sci. Eng.* **2013**, 11, 8
[30] C. Sacasa Castellanos, *University of Western Ontario - Electronic Thesis and Dissertation Repository.* Paper 1113. (2013). http://ir.lib.uwo.ca/etd/1113
[31] K. Kim, N. Jong, I. S. Suh, B.-K. Hur, C. Gyunlee, *J. Microbiol. Biotechnol*. **2002**, 12(6), 962–971
[32] C.-G. Lee, *Biotechnol. Bioprocess Eng.* **1999**, 4, 78-81.
[33] F. G. A. Fernandez, F. Garcia Camacho, J. A. Sanchez Perez, J. M. Fernandez Sevilla, and E. Molina Grima. *Biotechnol. Bioeng.* **1997**, 55 701-714
[34] G. A. Lutzu, M. Pisu, G. Cao, *Chem. Eng. J.* **2012**, 213, 203-213.
[35] A. Concas, M. Pisu, G. Cao, *Chem. Eng. J.* **2013**, 157, 297-303.
[36] S. Li, S. Luo, R. Guo, *Bioresour. Technol.* **2013**. 136, 267-272.
[37] K. Kumar, C. N. Dasgupta, B. Nayak, P, Lindblad, D. Das, *Bioresour. Technol.* **2011**. 102, 4945-4953.
[38] E. Jacob-Lopes, L. M. C. F. Lacerda, T. T. Franco, *Biochem. Eng. J.* **2008**, 40, 27-34.
[39] S.-H. Ho, C.-Y. Chen, J.-S. Chang, *Bioresour. Technol.* **2012**, 113, 244-252
[40] N. Hanagata, T. Takeuchi, Y., Fukuju, D. J. Barnes, I. Karube, *Phytochem.* **1992**, 31, 3345-3348.

Figure Legends

Fig. 1. Experimental set up for the photobioreactor,

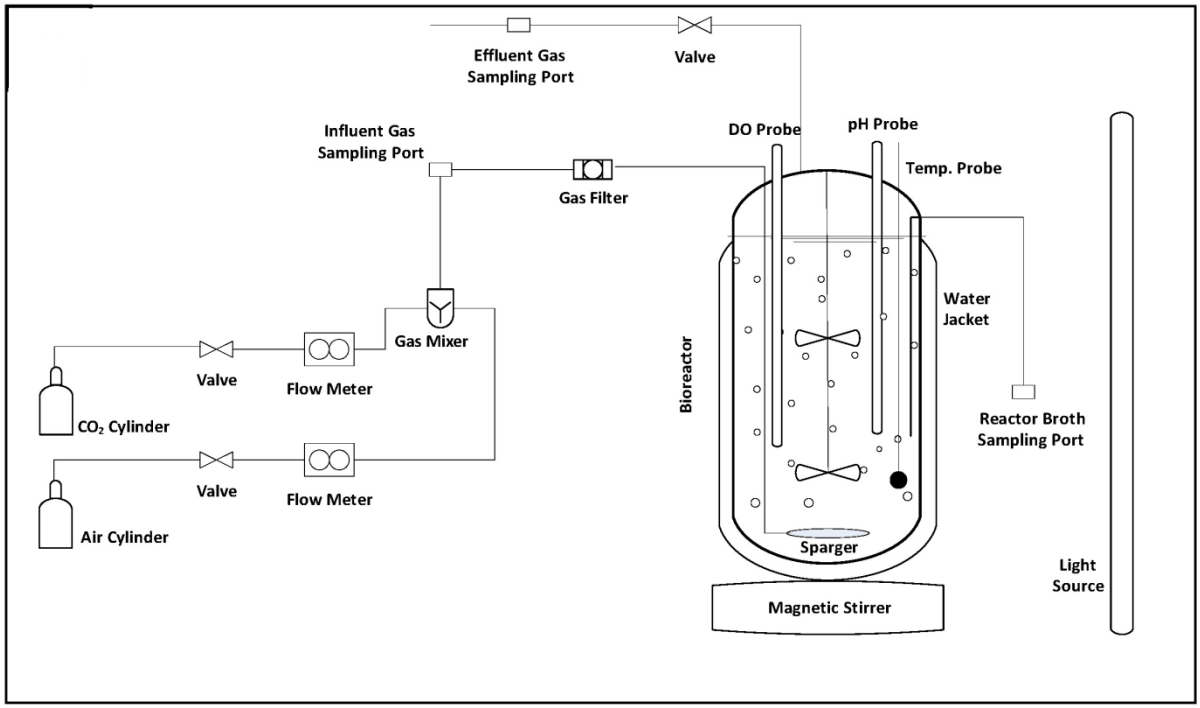

Fig. 2. Growth curve of microalgae cultivation at different light intensities.

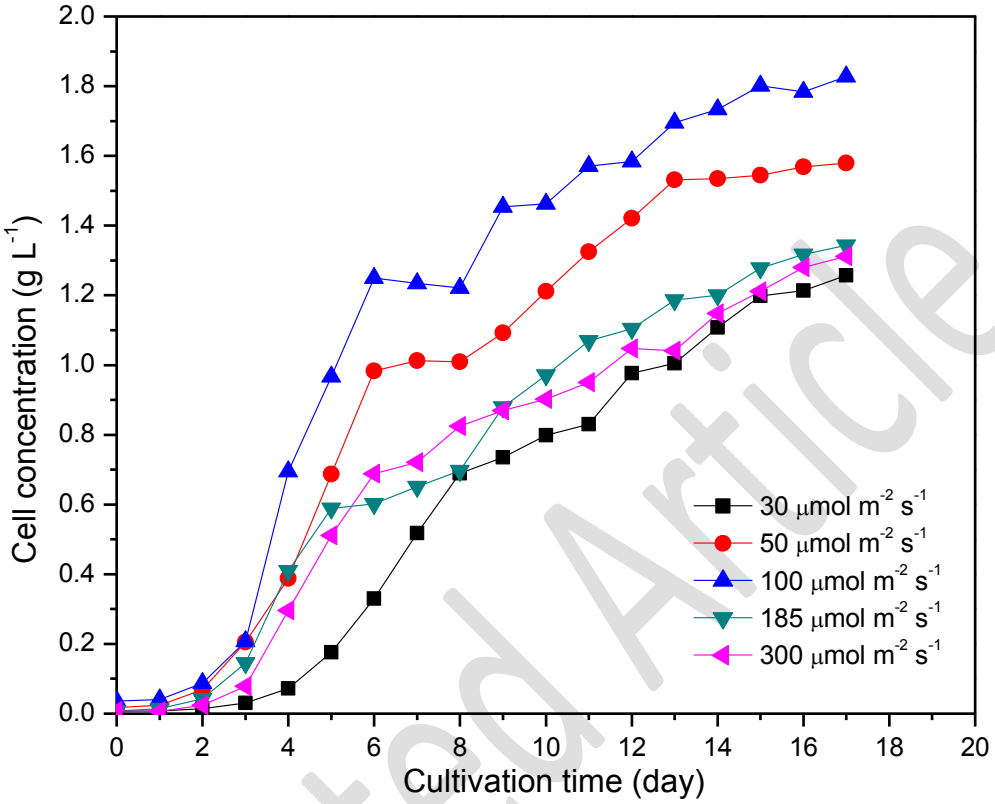

Fig. 3. pH evolution for experiments at different light intensities over time.

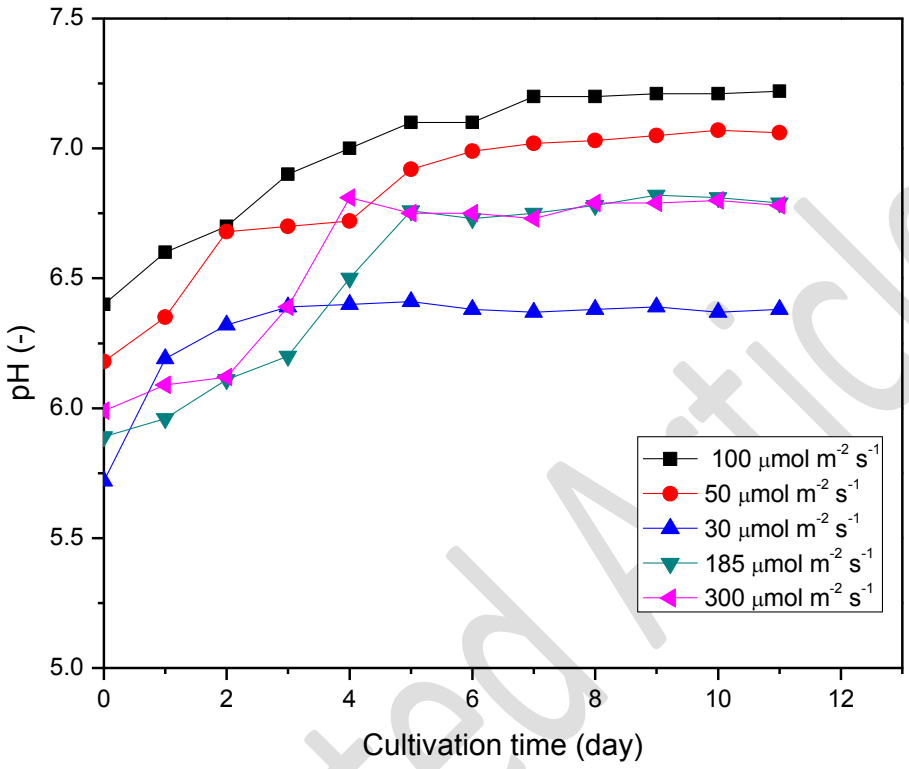

Fig. 4. Effect of biomass growth on pH in experiment with 100 µmol m^{-2} s^{-1} light intensity

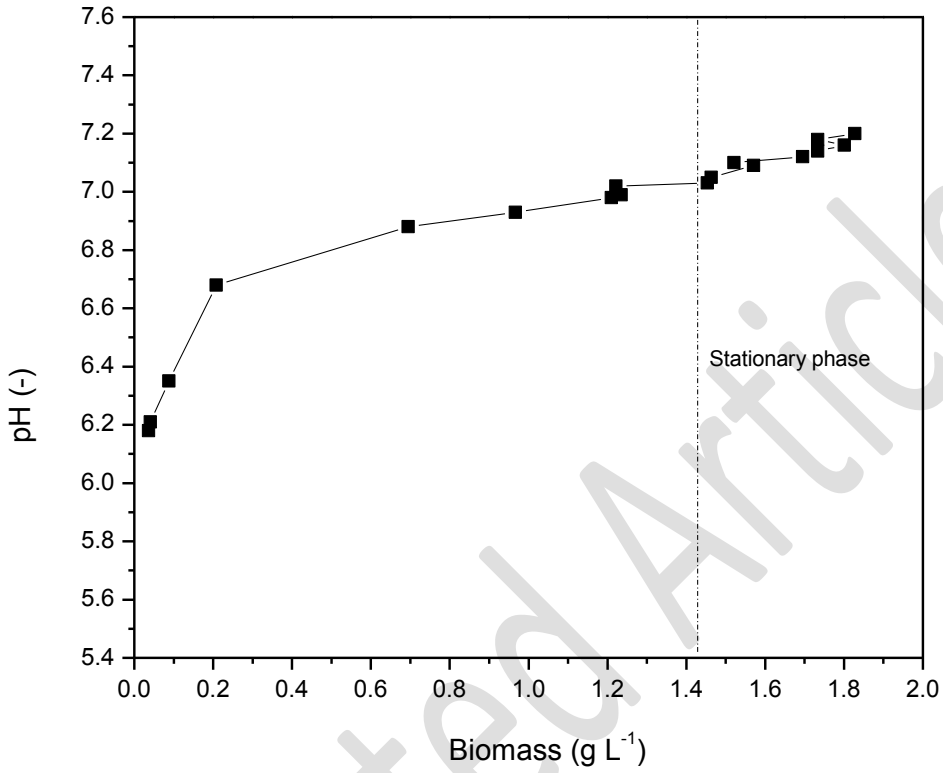

Fig. 5. Cell density at 6 days and 17 days (g L^{-1}) and pH trends when the alga was subjected to various light intensities.

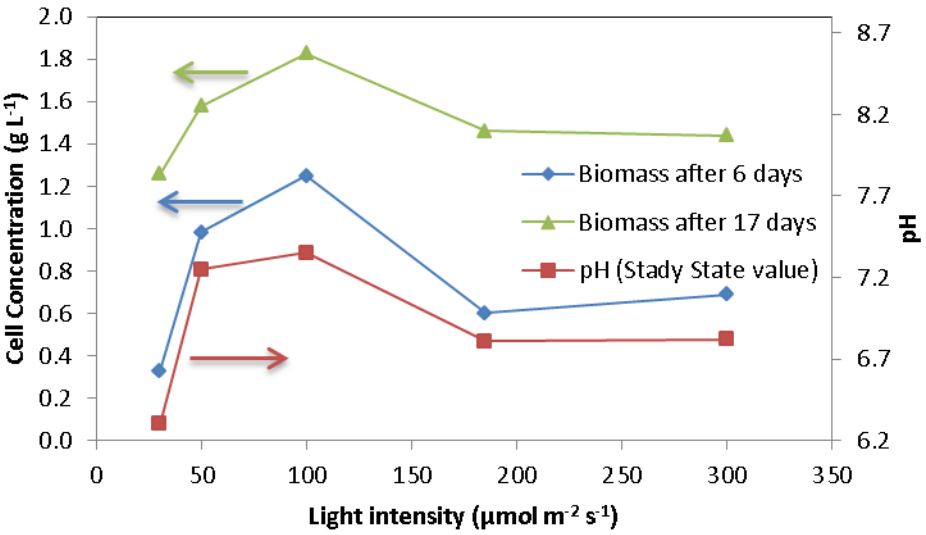

Fig. 6. Biomass concentration (g L^{-1}) and DO evolution over time (d), at light intensities of 50, 100 and 185 µmol m^{-2} s^{-1} for graphs A, B and C, respectively.

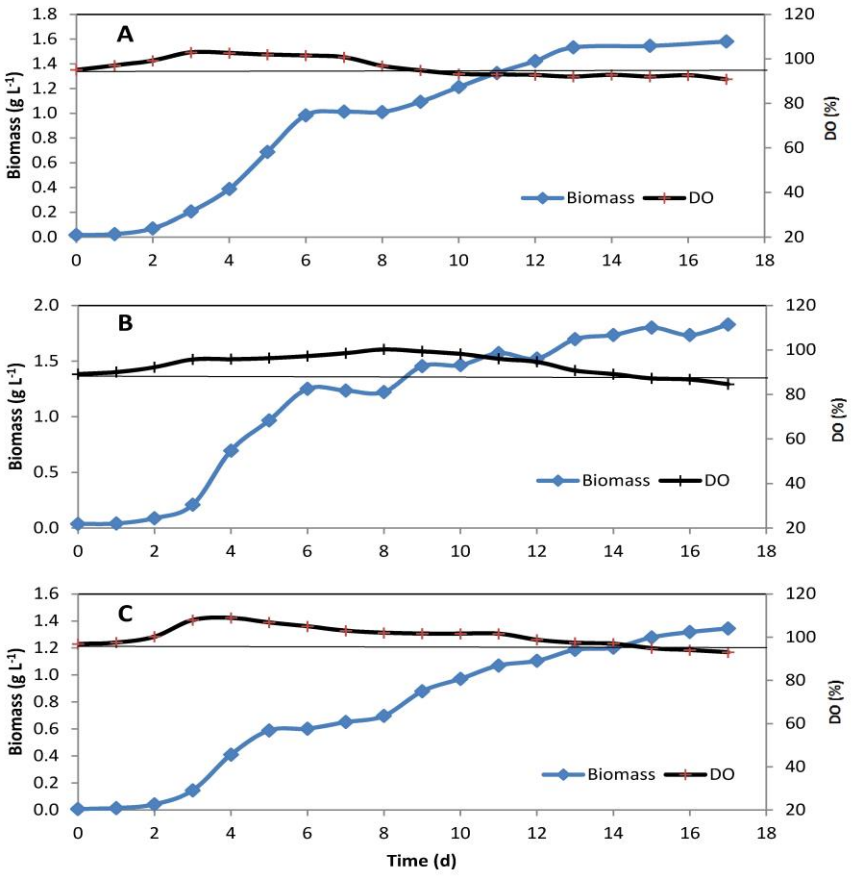

Figure 6. Biomass concentration (g L^{-1}) and DO evolution over time (d), at light intensities of 50, 100 and 185 µmol m^{-2} s^{-1} for graphs A, B and C, respectively.

Fig. 7. Effect of light intensity on specific growth rate (d^{-1}), productivity (g DW L^{-1} d^{-1}) and CO_2 biofixation (g CO_2 L^{-1} d^{-1}).

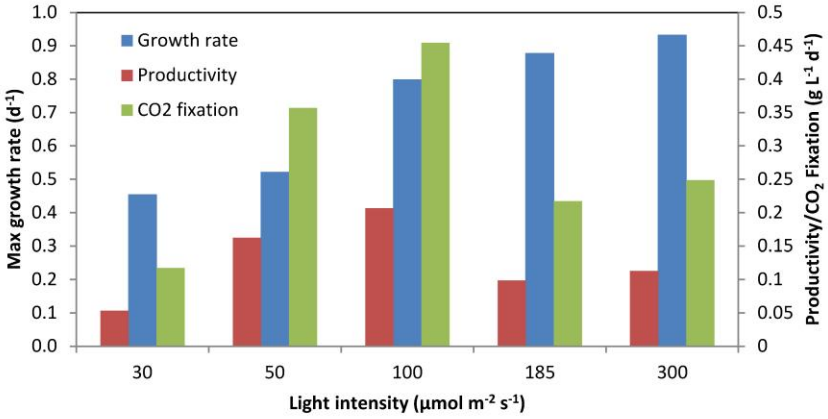

Figure 7. Effect of light intensity on specific growth rate (d^{-1}), productivity (g DW L^{-1} d^{-1}) and CO_2 biofixation (g CO_2 L^{-1} d^{-1}).

Tables and Table Legends

Table 1: Comparison of CO_2 fixation rate and optimum light intensity for different microalgae species.

Algae Species	Inlet CO_2% (%v/v)	Flow rate (vvm)	Light intensity (µmol m^{-2} s^{-1})	CO_2 Fixation rate (g $L^{-1}d^{-1}$)	References
Aphanothece microscopica N¨ageli	15	1	150	1.44	[5]
Chlorella vulgaris	4	-	80	0.148	[11]
Anabaena sp.	Air	0.2	900	1.45	[12]
Anabaena sp.	10	0.04	70	1.01	[13]
Anabaena sp.	Air	0.25	1625	1	[14]
Synechocystis aquatilis SI-2	10	0.05	-	3.3	[15]
Chlorella vulgaris	15	2	110	0.624	[16]
Chlorella vulgaris	10-13	-	1150	4.4	[17]
Chlorella vulgaris	1	0.5	165	6.24	[18]
Chlorococcum sp.	10	0.004	-	0.305	[19]
Dunaliella salina	3	-	80	0.091	[20]
Synechocystis sp.	10	0.4	1600	1.96	[21]
Chlorella sp.	2	0.25	300	7.83	[22]
Spirulina sp.	6	0.075	3200 lux	0.39	[23]
Scenedesmus obliquus	6	0.075	3200 lux	0.2	[23]
Chlorella kessleri	6	0.075	3200 lux	0.12	[23]
Spirulina platensis	5	-	3500 lux	0.319	[24]
Botrycoccus braunii	5	-	3500 lux	0.497	[24]
Chlorella vulgaris	5	-	3500 lux	0.252	[24]
Chlorella sp.	5	0.2	100	0.7	[25]

Table 2. Light enclosure set-up.

Experiment	Light intensity (µmol m^{-2} s^{-1})	Light enclosure configuration
Run 1	30	4 lamps on, at 10-cm distance
Run 2	50	8 lamps on, at 10-cm distance
Run 3	100	16 lamps on, at 10-cm distance
Run 4	185	16 lamps on, at 5-cm distance

Run 5	300	16 lamps on, at 1-cm distance

Table of Contents: Graphical Abstracts

Photobioreactor enclosed with unique light enclosure configuration, where the light intensities can be investigated accurately.