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Improvement of carbon dioxide removal through artificial light intensity and temperature by constructed green microalgae consortium in a vertical bubble column photobioreactor

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Aims: This study was aimed to demonstrate that environmental conditions, such as light intensity, photoperiodism and temperature, played a determining role in improving CO_2 removal and cellular propagation. Photosynthesis by microalgae in a direct CO_2 to biomass conversion in engineered systems such as photobioreactors, has been frequently used for CO_2 removal. The goal of this study was to obtain high CO_2 removal by green microalgae strains cultivated in a vertical bubble column photobioreactor.
Methodology and results: Constructed consortium containing *Chlorella* sp., *Scenedesmus obliquus*, and *Ankistrodesmus* sp. were cultured in temperatures (°C) of 25, 30, 25; light intensities (lux) of 2500, 4000 and photoperiodisms (light/dark; hour) of 24/0, 16/8, and 12/12. The experiment demonstrated that microalgae was capable of tolerating up to 7% CO_2 concentrations under variation of light intensities, photoperiodisms, and temperature conditions. Synergetic of three microalgae capable of utilizing CO_2 and transformed it to become biomass. The result also showed that growth was best at light intensity of 4000 lux for 16 h a day and temperature of 30 °C. The maximum growth rate (μ) of 0.38 per day was obtained from culture injected with 5% CO_2 concentration.
Conclusion, significance and impact study: The CO_2 removal efficiency (%) was 49.02, whereas CO_2 utilization efficiency (%), carbon dioxide transfer rate (CTR; gCO_2/L.h) and carbon dioxide fixation rate (qCO_2/h) were 15.15, 101.29 and 42.02, respectively. Biofixation of CO_2 by the constructed consortium has recently gained renewed interest as a promising strategy for CO_2 mitigation.

Keywords: light intensity, photoperiodism, CO_2 removal, CO_2 fixation, biomitigation

INTRODUCTION

Carbon fixation by photoautotrophic microalgae has the potential to diminish the release of CO_2 into the atmosphere and helping to alleviate the trend toward global warming. In order to obtain a higher CO_2 removal efficiency, high-density cultivation was performed. Under these conditions, more CO_2 will be consumed by the microalgae.

Association of microalgae culture seems a promising technology for sustainable algal biomass and biological removal of carbon dioxide. Our previous study concluded that single culture as well as mixed culture could tolerate live and grew well in high CO_2 concentration due to indications of dry biomass and growth rate. However, mixed culture has higher removal efficiency of CO_2 than single culture of Chlorella sp. *Scenedesmus obliquus*, and *Ankistrodesmus* sp. (Rinanti *et al.*, 2013).

The most important environmental factor for the growth of photosynthetic unicellular microalgae is light. Light provides energy for the photoautotrophic process. Concerning light intensity, operational issues are not yet clear cut. On one hand, the use of sunlight is cheaper but light cycle cannot be controlled, which often precludes higher biomass productivities. However, the quality and duration of light to which the algae are exposed in the natural environment is not always available at the best conditions for the optimum growth of the algae.

On the other hand, artificially illuminated photo-bioreactors are typically expensive. The intensity and utilization efficiency of the light supplied are thus of crucial importance in microalgae bioreactors (Kumar *et al.*, 2010). The growth of microalgae, which is also photosynthetic, is controlled by both the spectral quality and quantity of the light source and the length of daylight (Striebal *et al.*, 2009). Photosynthesis is the production of organic compounds (including carbohydrates, proteins, and lipids) using inorganic substances (such as carbon dioxide and water). This process involves the conversion of light energy into chemical energy. Chemical

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substances called pigments absorb light. The main pigment used to absorb light in photosynthesis is chlorophyll. Carbon dioxide is absorbed for photosynthesis.

For the CO_2 fixation and biomass production optimum light intensity is necessary. Below the optimum light intensity, light becomes the limiting factor for the microalgae productivity. On the other hand, exposure of cells to long period with high light intensity causes photoinhibition (Rubio *et al.*, 2003). Masojideck *et al.* (2011) also described phenomenon of photoinhibition. Under prolonged irradiation at a supra-optimal level, photosynthetic rates usually decline from their light-saturated values. A further rise in light intensity to above 8000 lux did not make much difference to either the growth rate or the dry weight of the microalgae, suggesting that a light saturation point had been reached. Saturation light intensity roughly varies from 30 to 45 W/m2 (140–210 $\mu E/m^2.s^1$) with a good estimation. For example, according to Hanagata *et al.* (1992) saturation light intensity of *Chlorella* sp. and *Scendesmus* sp. is around 200 $\mu E/m^2.s^1$. The ratio of light to dark (or low-intensity light) periods in a cycle is crucial for microalgae productivity (Muñoz and Guieysse, 2006).

Temperature is the most important limiting factor, after light, for culturing algae in both close and open outdoor systems that regulate cellular, morphological and physiological responses of microalgae (Vonshak *et al.*, 2001; Carlozzi, 2003; Moheimani, 2005; Chisti, 2007). Higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of microalgae growth (Muñoz and Guieysse, 2006). The optimum growth temperature of most microalgae is in the range of 20-30 °C (Wang *et al.*, 2008). When the temperature is much lower or much higher than the optimum, specific growth rate of microalgae is reduced (Madigan *et al.*, 2000; Thébault *et al.*, 2003).

The goal of the study was to obtain high CO_2 removal by green microalgae consortium cultivated in a vertical bubble column photobioreactor that are capable of tolerating up to 7% CO_2 (gas phase) concentrations under variation of light intensity, light photoperiodism (light/dark), and temperature.

MATERIALS AND METHODS

Microorganisms and growth medium

The microalgae consortium consists of 3 green microalgae *Chlorella* sp., *Scenedesmus obliquus* and *Ankistrodesmus* sp. with the same density ratio, isolated from Bojong Soang Municipal Waste Water Treatment Plant. Activation and cultivation carried out in sterilized artificial medium Phovasoli Haematococcus Media (PHM) (Provasoli and Pintner, 1960), the pH 7 + 0.5.

Cultivation and growth conditions in a vertical photobioreactor

A vertical photobioreactor made of glass with a capacity of 10 L containing 8 L of artificial growth medium for microalgae consortium cultivation. To achieve the objectives of this study, photobioreactor equipped with a source of gas from CO_2 gas cylinder and aerator. Pure CO_2 was injected from the bottom of the column to allow gas mixing with the medium. Sparger attached at the bottom of the reactor to convert the gas into small bubbles (Chai *et al.*, 2010). Sparging with microbubble allow thorough mixing, CO_2 mass transfer and also removes O_2 produced during photosynthesis. To observe the influence of light intensity, light photoperiodism and temperature on growth and CO_2 fixation, microalgae consortium was cultivated at 2,500 lux and 4,000 lux light intensities. Light intensities were provided by white fluorescent lamps and light photoperiodism (light/dark; hour) were designed to be 24/0, 16/8 and 12/12. Microalgae consortium was then cultivated at 25 °C, 30 °C and 35 °C. During the process of growth in photobioreactor, 2%, 5 %, 7% pure CO_2 was continuously supplied trough the bottom of the photobioreactor, with a flow rate 48 L/h.

Measurement of growth respond

Dry weight cell biomass was obtained by evaporating the liquid in the cell culture. A total of 100 mL culture tube inserted into centrifuges, and then centrifuged at 3,500 rpm for 10 min (Weldy and Huesemann, 2007). Supernatant was then removed from the tube pasta until just earned cells. Pasta cells were then put into a Petri dish that had previously been weighed (x). Samples were put in the oven with a temperature of 105 °C for one night to get a constant weight (y), and then stored in a desiccator for 30 min before re-weighed. Biomass (dry weight) according to Torzillo *et al.* (1991) calculated by the formula: Dry weight (X; mg) = y (mg) - x (mg). Specific growth rate (μ; /d) was calculated as follows:

$$\mu = \frac{1}{X}.\frac{dX}{dt} \qquad \text{(Eq.1)}$$

Measurement of CO_2 removal

Concentration of CO_2 in a series of photobioreactor system was measured 2 (two) times a day, using Combination Portable Gas Detector Model RX-515 RIKEN. Measurements were performed to determine changes in the concentration of CO_2 in the gas holder with time, whereas the concentration of CO_2 dissolved in the culture medium was measured once daily by using acidity alkalinity method to know the solubility of CO_2 in the culture medium.

CO_2 removal efficiency is the proportion of the absorbed CO_2 concentration by the photobioreactor system to CO_2 that was supplied.

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CO_2 removal efficiency= $\frac{\text{influent of } CO_2 - \text{effluent of } CO_2}{\text{influent of } CO_2} \times 100\%$

(Eq.2)

Carbon Transfer Rate (CTR; $gCO_2/L.h$) is the amount of CO_2 that is transferred in the medium volume and required by the cell metabolism for a unit of time (Dianursanti, 2012).

$$CTR = \Delta(yCO_2).\ \alpha CO_2 \quad \text{(Eq.3)}$$

Where,
αCO_2= a constant of CO_2 that contains a fixed number of temperature and pressure, airflow superficial velocity; $\Delta(yCO_2)$ = the concentration change of CO_2 in and out of the reactor by the incoming CO_2 concentration, multiplied by 100%

$$\propto_{CO_2} = \frac{U_g\ .\ A.\ M_{CO_2}.P}{V_{med}\ .R.T}$$

(Eq. 4)

Where, Ug = superficial gas velocity i.e discharge gas fed per reactor cross-sectional area (m/h); A = surface area of the photobioreactor facing or exposed to light (m2); MCO2 = relative molecular mass of CO_2 (44 mol); P = operating pressure (1 atm); V = volume of medium (L); R = Rydberg constant (0.08205 L.atm/mol.K); T = operating temperature (0 K).

Carbon fixation rate as specific CO_2 transfer rate (qCO_2; gCO_2.g/cell.h) is the rate of CO_2 that is transferred in a medium volume due to the activity of biological life within a unit of time.

$$q_{CO_2} = \frac{\Delta y_{CO_2}.\propto_{CO_2}}{X}$$

(Eq.5)

Where, X = cell dry weight per unit volume (g/L).
An approximate formula ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) suggested by Grobbelaar (2004) was used to make an expected estimate of the dry biomass yield. CO_2 utilization efficiency was determined by using the following equation (Ryu *et al.*, 2009):

CO_2 utilization efficiency (%) = 0.57 x P x [(44/12) / V_{CO2}] x 100

(Eq.6)

Where, 0.57 is the carbon content of the dry weight cell (gcarbon/gbiomass), 44 and 12 are the molecular weights of carbon dioxide and carbon, P is the productivity (g biomass/L.h), respectively, and V is the aeration rate of CO_2 supplied to the microalgae culture medium (g CO_2/L.h).

RESULTS AND DISCUSSIONS

Effect of light intensity, photoperiodism and temperature were discussed in term of growth response and its relation with CO_2 removal.

Effect of light intensities

At different experimental irradiances, 4,000 lux was found to be the optimum light intensity for biomass production. Whereas a higher light intensity of 5,000 lux caused photo inhibition, therefore microalgae consortium could not grow well since day 1 to day 3 (data not shown). The highest biomass (2.2 g/L), and growth rate (0.094/doubling day) was found at 4,000 lux light intensity (Figure 1).

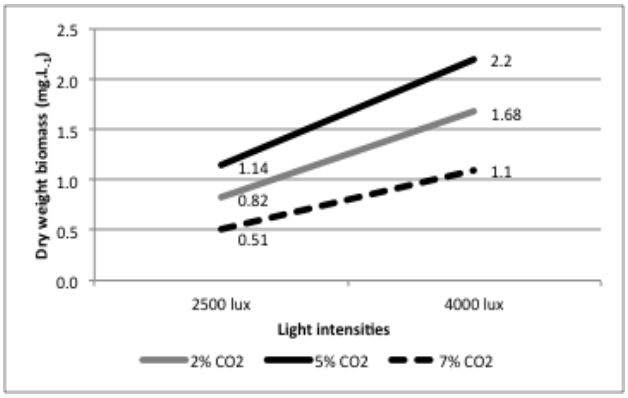

Figure 1: Dry weight biomass of microalgae consortium as a result of light intensities.

It means the biomass increased 2-fold highest compare to cultures in conditions of 2,500 lux. Each type of microalgae requires a certain light intensity for maximum growth (Vonshak *et al.*, 2001). Light intensity played an important role in the excitation of electrons contained in photosystem so photosynthesis could do (Beardall and Raven, 2011). Therefore, the research on the effects of light photoperiodism and temperature would work at 4,000 lux light intensity.

It was crucial to select the optimum cell concentration or optimum dry weight biomass for the efficient CO_2 removal. Below the optimum cell concentration, not all the light energy was captured by the cells while at above the optimum cell concentration, a larger proportion of the cell were in the dark due to self-shading (Zhang *et al.*, 2001). Increasing dry weight biomass at 4,000 lux light intensity was followed by increasing 2-fold CO_2 removal efficiency compare to CO_2 removal efficiency in culture at low light intensity (Table 1). Microalgae density and volume cultivation determine how much light intensity was needed. All algae could take up CO_2 by diffusion, and

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Table 1: Average of CO_2 removal efficiency, carbon transfer rate and carbon fixation rate as a result of variety light intensities

Light intensities	CO_2 concentration	CO_2 removal efficiency (%)	Carbon transfer rate (gCO_2/L.h)	Carbon fixation rate (/h)
2,500 lux	2%	16.05	40.43	32.62
	5%	19.03	35.32	25.87
	7%	10.13	24.78	17.90
4,000 lux	2%	33.58	70.72	39.50
	5%	39.03	81.53	38.39
	7%	18.05	39.85	24.48

many had active carbon uptake systems which could take up bicarbonate (HCO_3^-). However, microalgae could not take up CO_3^{2-} ions (Badger, 2003).

Figure 2 showed that at a concentration of 2% and 5% CO_2, CO_2 utilization efficiency increased, while it was not working in the concentration of 7% CO_2. It was happened because the compound bicarbonate (HCO_3^-) at a concentration of 2% and 5% CO_2 could still be used by the microalgae to be converted into biomass with the help of CA (carbonic anhydrase), whereas CA activity was decreased in culture supplied by 7% CO_2 so that the effectiveness of the use of bicarbonate compounds catalysed by CA began to decrease.

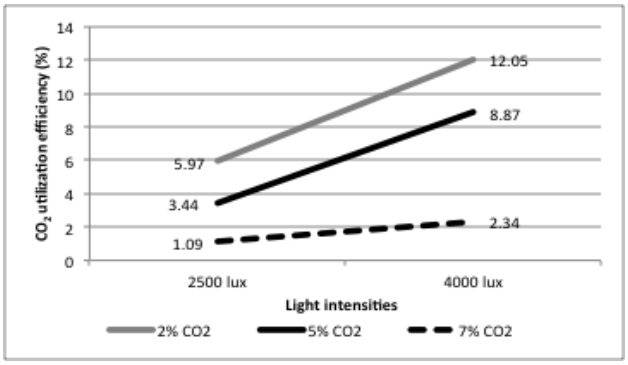

Figure 2: CO_2 utilization efficiency of microalgae consortium as a result of light intensities.

The distribution of efficient light throughout the photobioreactor affected the CTR and qCO_2 as described in Table 1. Such coefficient is influenced by the superficial velocity (Eq. 4), where superficial velocity (Ug) is the gas volumetric rate divided by the cross-sectional area reactor. In this study, the value of the superficial velocity was 652.23 dm/d or 27.18 dm/h.

Based on Dianursanti (2012), the superficial velocity was ideally in the range of 12-120 dm/h. Rated 27.18 dm/h still in that range, meaning that the use of superficial velocity on the value would not cause a significant change in the volume of the reactor. The researchers Singh and Majumder (2010), suggested that the superficial velocity under 180 dm/h and the diameter of the water affects the diameter of the bubble gas sparger. High superficial velocity can increase the gas hold-up and bubble surface area (small bubble diameter). Small bubble size caused mass transfer of CO_2 working well in this growth medium.

CO_2 fixation by microalgae consortium in a vertical bubble column photobioreactor is first marked by a difference in concentration of CO_2 that input into the reactor and the concentration of CO_2 coming out of the reactor. The difference in CO_2 concentration shows that there is a transfer of CO_2 from the air into the microalgae cultivation media. The rate of CO_2 fixation by microalgae qCO_2 is CO_2 that is transferred to the medium due to microbiological activity in a unit of time. Carbon fixation rate (qCO_2) value can be determined based on the CTR values divided by the dry biomass of microalgae.

Table 1 also shows qCO_2 value was inversely proportional to the production of dry biomass produced by microalgae. The value of qCO_2 is a comparison between the amount of CO_2 transferred to the medium and the amount of biomass produced. The greater the value of dry biomass of microalgae, the smaller the value qCO_2. It was represented the ability of CO_2 fixation by microalgae consortium which was grow well in 4,000 lux light intensity conditions. The efficient distribution of light throughout the photobioreactor affected the carbon dioxide utilization rates.

Effect of light photoperiodism

Figure 3 showed the difference of light photoperiodism conditions obtained in different dry weight of biomass. Highest biomass dry weight obtained at the condition of the light/dark (16/8), in a culture supplied by 5% CO_2. The increase in CO_2 concentration from 2% to 5% did not show an increase in dry weight biomass significantly, in dark light conditions (24/0), (16/8), and (12/12). However, the dry weight biomass decreased in all cultures supplied by 7% CO_2, light dark conditions (12/12). This showed that, light dark photoperiodism (24/0) and (16/8) did not cause the increase in dry weight biomass in the culture supplied by 2% and 5% CO_2 significantly.

Ryu *et al.* (2009) analyzed the impact of CO_2 concentration in vertical tubular reactors without controlling the temperature of the system. According to their findings, a maximum cell concentration of 2.02 g/L was found at 5% CO_2 and the minimum cell concentration of 1.16 g/L was found at 0.5% CO_2 mixed in air. They suggested keeping the CO_2 concentration lower than 5% because higher CO_2 concentrations might inhibit microalgae growth. The other researchers Chiu *et al.*

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(2009) demonstrated that the CO_2 removal efficiency in the porous centric-tube photobioreactor is 45 and 52% was higher than those in the bubble column and centric-tube photobioreactors, respectively.

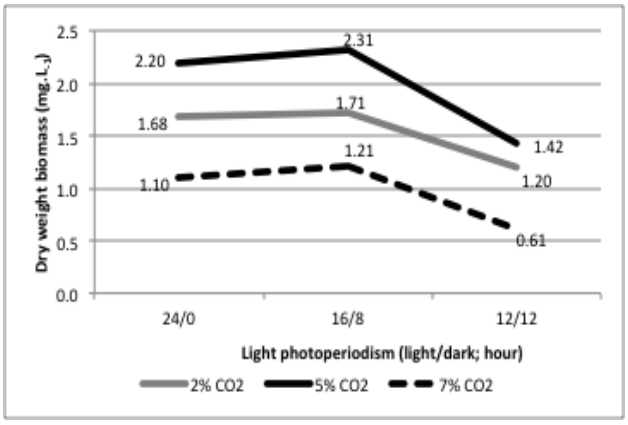

Figure 3: Dry weight biomass of microalgae consortium as a result of light photoperiodism.

Table 2 showed that same thing with biomass dry weight, light dark conditions (24/0) and (16/8) did not show the increase in CO_2 removal efficiency significantly by increasing 2% to 5% CO_2 concentration. However, in the light-dark conditions (16/8) obtained CO_2 removal efficiency 2-fold higher than in dark light conditions (12/12). This suggested that the removal of CO_2 in microalgae photobioreactor system was affected by the length of the sufficient light. For efficiency of energy sources derived from artificial light, the next experiments did not use dark light conditions (24/0) but instead, using light dark conditions (16/8).

CO_2 utilization efficiency differences between light and dark conditions can be seen in Figure 4 In long dark periods (12/12), microalgae could not do biomass synthesis process completely. Cell respiration was more dominant so that the culture medium became saturated with carbonate compounds that were not utilized by microalgae. As a result, the process of transfer of CO_2 into the cell microalgae decreased. In this experiment, CO_2 utilization efficiency in light-dark conditions (12/12) only reaches half its efficiency that showed in the shorter dark conditions (8 h of dark). This was the case in all variation of CO_2 concentrations.

Table 2: Average of CO_2 removal efficiency, carbon transfer rate and carbon fixation rate as a result of variety light photoperiodism.

Light photoperiodism (light/dark; h)	CO_2 concentration	CO_2 removal efficiency (%)	Carbon transfer rate (gCO_2/L.h)	Carbon fixation rate (/h)
24/0	2%	33.58	70.72	39.50
	5%	39.03	81.53	38.39
	7%	18.05	39.85	24.48
16/8	2%	36.33	76.35	36.52
	5%	43.13	90.24	39.84
	7%	21.03	44.63	38.39
12/12	2%	20.49	43.46	29.90
	5%	22.80	48.32	20.23
	7%	11.02	23.39	29.70

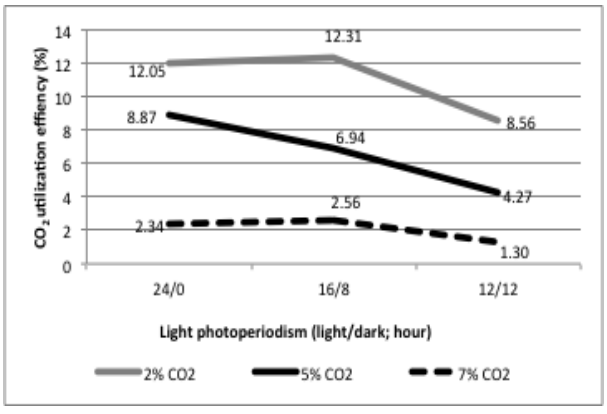

Figure 4: CO_2 utilization efficiency of microalgae consortium as a result of light photoperiodism.

CTR values increased since the beginning of the growth period, when the concentration of CO_2 in the culture medium was below the threshold of saturation, so the CO_2 gas more soluble in the culture medium (Table 2). Trend curves CTR during cultivation is look like shown in Figure 5. In addition, the increase of cell density and dry weight biomass enhanced the absorption of gas dissolved in the form of HCO_3^- by microalgae. CTR tended to decrease over time at day 10 due to the imbalance between increasing of cells densities and CO_2 biofixation. This condition eventually caused biomass production constant and finally decreased.

Table 2 also shows the values qCO_2 inversely proportional to the value of biomass dry weight during cultivation. The higher growth of microalgae correlated with the smaller value of qCO_2. It was happened because the increased biomass production resulted in less CO_2 available that could be fixed by the cells. Therefore the rate of CO_2 fixation decreased.

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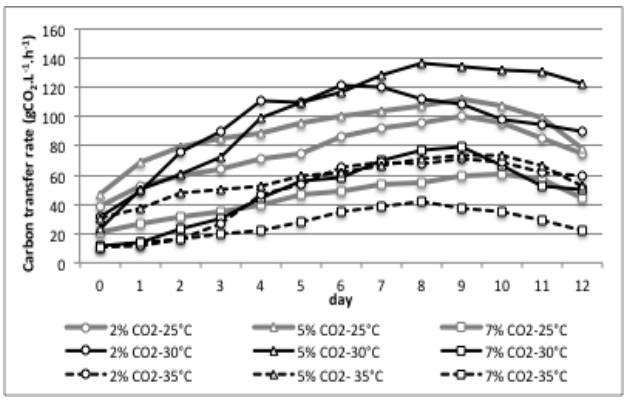

Figure 5: Carbon transfer rate as a result of variety temperature.

The same observation applied to the value of the CTR. The average value of qCO_2 in condition light/ (24/0) of 39 h did not differ significantly compare with light/dark of (16/8). While in condition of light/ dark (12/12) the values of qCO_2 was only 29/h. Differences in qCO_2 values for both conditions was caused by the fact that microalgae did not take carbon source of the CO_2 entering the reactor in dark conditions.

The average CTR at light-dark conditions (16/8) reached 90 g/L.h. The value did not differ significantly compare with light-dark conditions (24/0). However in light-dark conditions (12/12) the value of CTR was observed only 48 g/L.h (Table 2). CTR values for the two conditions were significantly different because in dark conditions microalgae was not experiencing the full growth cycle. Respiration processes occurred predominantly intra-cellular, so that transfer of carbon from the CO_2 entering the reactor greatly reduced. This phenomenon led to the transfer of CO_2 in the light-dark conditions (12/12) was observed to be smaller than in the light-dark conditions either (24/0) or (16/8).

Until the end of the study, culture supplied with 7% CO_2 gave the most unfavorable response compared with 2% and 5% CO_2. High CO_2 concentrations (>5%) generally become toxic to microalgae, presumably because the medium becomes acidic from carbonic acid.

Effect of increasing temperature

Biomass productivity of constructed consortium was evaluated at various temperatures (25 °C, 30 °C, and 35 °C) for a period of 12 days. Growth analysis of cultures grown at different temperatures showed significant differences (p < 0.05) in growth pattern. Maximum biomass concentration (as dry weight) ie. 2.70 g/L was observed at temperature 30 °C and least i.e. 2.11 g/L was found at temperature 25 °C (Figure 6). Growth curve of cultures at various temperatures revealed that green microalgae species had a wide range of temperature tolerance, ranging from 20 °C to 40 °C. In this experiment, cultures at 40 °C did not show exponential growth. The growth was almost negligible (data not recorded). The maximum growth rate ie. 0.38/ doubling day was observed at 30 °C, but with further increase in temperature reduction in growth rate was observed. At 35 °C culture showed 0.12/doubling day which was almost 30% slower as compare to growth rate at 30 °C. It was proved that extreme temperatures did not support the growth of mix culture.

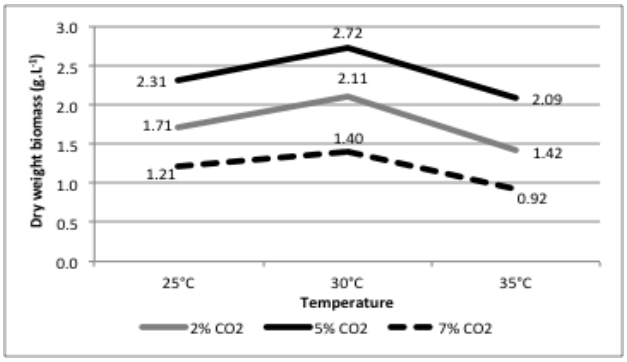

Figure 6: Dry weight biomass of microalgae consortium as a result of temperature.

The experiment also demonstrated that there were increased in dry weight biomass and CO_2 removal efficiency (Figure 6 and Table 3) ranging from 2 to 2.5 times higher than the initial conditions (Figure 1 and Table 1) in all variations of CO_2 concentrations. CO_2 removal efficiency by microalgae was influenced by several factors, such as CO_2 concentration and flow rate (Ryu *et al.*, 2009), light intensity (Perner-Nochta and Posten, 2007), lightfotoperiodism (Lopes *et al.*, 2008), cell density (Jiang *et al.*, 2011), temperature (Chinnasamy, 2009), and the type of reactor (Kumar *et al.*, 2010).

Increasing temperature up to 30 °C also increased CO_2 utilization efficiency to over 5-fold (Figure 7) compared to initial conditions (Figure 2). However at a temperature of 35 °C efficiency was decreased.

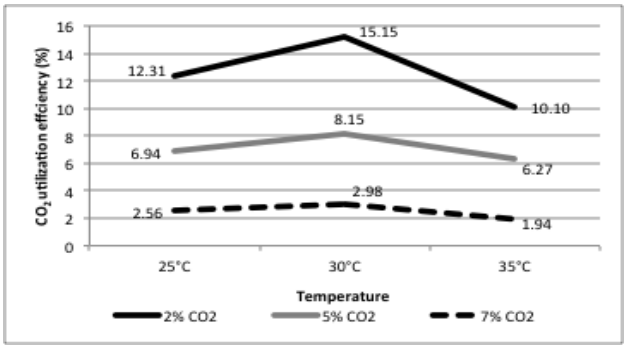

Figure 7: CO_2 utilization efficiency of microalgae consortium as a result of temperature.

As temperature increased, the rate of photosynthesis increased more rapidly until the optimum temperature reached. Above the optimum temperature the rate of

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Table 3: Average of CO_2 removal efficiency, carbon transfer rate and carbon fixation rate as a result of variety temperature.

Temperature	CO_2 concentration	CO_2 removal efficiency (%)	Carbon transfer rate (gCO_2/L.h)	Carbon fixation rate (/h)
25 °C	2%	36.33	76.35	36.90
	5%	43.13	90.24	35.54
	7%	21.03	44.63	30.70
30 °C	2%	42.95	93.17	40.08
	5%	49.02	101.29	42.02
	7%	23.18	48.55	38.21
35 °C	2%	23.94	48.46	34.22
	5%	28.13	57.30	22.36
	7%	13.07	26.84	24.20

photosynthesis dropped significantly. This was because of fixation of CO_2 that was catalyzed by enzymes, which faster at higher temperatures. Nevertheless the enzyme denatured in high temperature. This explained why at 35 °C the CO_2 utilization efficiency was decreased (Figure 7).

Carbon fixation rate also slower than culture condition at 30 °C (Figure 8) and then at 40 °C the growth was almost negligible (data not recorded). Although average of CTR values obtained from 30 °C conditions seemed to be higher than 25 °C condition, however the difference was not significant (Table 3). CTR values obtained at a temperature of 30 °C reached 2-fold higher than the CTR value obtained at 35 °C conditions (Table 3). Compared to the initial conditions (Table 1, 2,500 lux), the CTR values obtained in all variation of CO_2 concentrations, increased by approximately 2-fold (occurred in culture which were supplied by 2% and 5% CO_2) and reached 2.8-fold obtained in culture which was supplied with 5% CO_2. This proved that the value of CTR was affected by the temperature in the photobioreactor. However the density of microalgae consortium did not give any effect. Despite the growth of microalgae increased, carbon transfer in the growth medium was not inhibited. Trend curves CTR during cultivation is look like shown in Figure 5.

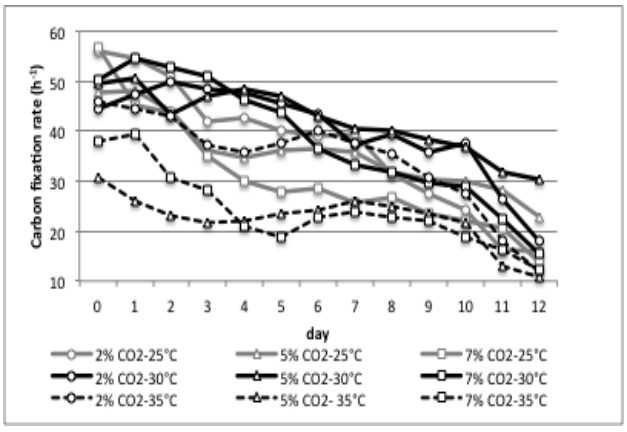

Figure 8: Carbon fixation rate as a result of variety temperature.

The optimum temperature for the growth of microalgae was 25-400 °C (Hanagata *et al.*, 1992; Vonshak *et al.*, 2001; Chinnasamy, 2009). Temperature affected the processes of physics, chemistry and biology that were happening in the microalgae cells. Increasing the temperature up to a certain limit would stimulate the activity of the molecule, increasing the rate of diffusion and the rate of photosynthesis (Badger, 2003).

Although at 7% CO_2 concentration all the results of this study showed lower value than the culture that were supplied by 2% and 5% of CO_2, however microalgae consortium remained able to growth and utilize the high CO_2 concentration (7% CO_2).

CONCLUSION

Controlling environmental parameters could improve the ability of microalgae to remove CO_2. CO_2 removal efficiency was highest when microalgae consortium cultivated in 4,000 lux light intensity, periods of light/dark (16/8), and temperature 30 °C. Microalgae consortium demonstrated optimum capacity to remove CO_2 at 5% CO_2 supplied. This was evidenced by dry weight of biomass which was 2.5 times higher, CO_2 removal efficiency above 2.5 times higher and the CO_2 utilization efficiency over 5 times higher. In addition, carbon transfer rate also increased. All results were compared with initial condition (2,500 lux, light/dark (24/0), 25 °C). Nevertheless carbon fixation rate did not increase significantly. It is interesting to see if supplying a higher CO_2 concentration would also increase capacity of CO_2 removal by microalgae consortium. This remains to be investigated in further research.

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