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

Astri Rinanti¹, Edwan Kardena¹*, Dea Indriani Astuti², Kania Dewi¹

¹Faculty of Civil and Environmental Engineering, Bandung Institute of Technology, Indonesia. Jl. Ganesha No. 10, Bandung 40132.
²School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia.Jl. Ganesha No. 10, Bandung 40132.

Email: kardena@pusat.itb.ac.id

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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 CO₂ removal and cellular propagation. Photosynthesis by microalgae in a direct CO₂ to biomass conversion in engineered systems such as photobioreactors, has been frequently used for CO₂ removal. The goal of this study was to obtain high CO₂ 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% CO₂ concentrations under variation of light intensities, photoperiodisms, and temperature conditions. Synergetic of three microalgae capable of utilizing CO₂ 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% CO₂ concentration.

Conclusion, significance and impact study: The CO₂ removal efficiency (%) was 49.02, whereas CO₂ utilization efficiency (%), carbon dioxide transfer rate (CTR; gCO₂/L·h) and carbon dioxide fixation rate (qCO₂/h) were 15.15, 101.29 and 42.02, respectively. Biofixation of CO₂ by the constructed consortium has recently gained renewed interest as a promising strategy for CO₂ mitigation.

Keywords: light intensity, photoperiodism, CO₂ removal, CO₂ fixation, biomitigation

INTRODUCTION

Carbon fixation by photoautotrophic microalgae has the potential to diminish the release of CO₂ into the atmosphere and helping to alleviate the trend toward global warming. In order to obtain a higher CO₂ removal efficiency, high-density cultivation was performed. Under these conditions, more CO₂ 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 CO₂ concentration due to indications of dry biomass and growth rate. However, mixed culture has higher removal efficiency of CO₂ 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 photobioreactors 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
substances called pigments absorb light. The main pigment used to absorb light in photosynthesis is chlorophyll. Carbon dioxide is absorbed for photosynthesis.

For the CO₂ fixation and biomass production optimum light intensity is necessary. Below the optimum light intensity, light becomes the limiting factor for the microalgal 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 µE/m².s)¹ with a good estimation. For example, according to Hanagata et al. (1992) saturation light intensity of Chlorella sp. and Scenedesmus sp. is around 200 µE/m².s. The ratio of light to dark (or low-intensity light) periods in a cycle is crucial for microalgal productivity (Muñoz and Güeyssé, 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 microalgal growth (Muñoz and Güeyssé, 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 CO₂ removal by green microalgal consortium cultivated in a vertical bubble column photobioreactor that are capable of tolerating up to 7% CO₂ (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 CO₂ gas cylinder and aerator. Pure CO₂ 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, CO₂ mass transfer and also removes O₂ produced during photosynthesis. To observe the influence of light intensity, light photoperiodism and temperature on growth and CO₂ 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. Microalgal consortium was then cultivated at 25 °C, 30 °C and 35 °C. During the process of growth in photobioreactor, 2%, 5%, 7% pure CO₂ was continuously supplied through 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:

\[
µ = \frac{1}{x} \frac{dx}{dt}
\]  

(Eq.1)

**Measurement of CO₂ removal**

Concentration of CO₂ 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 CO₂ in the gas holder with time, whereas the concentration of CO₂ dissolved in the culture medium was measured once daily by using acidity alkalinity method to know the solubility of CO₂ in the culture medium.

CO₂ removal efficiency is the proportion of the absorbed CO₂ concentration by the photobioreactor system to CO₂ that was supplied.
CO₂ removal efficiency = \frac{\text{influent of CO₂} - \text{effluent of CO₂} \times 100}{\text{influent of CO₂}}

\text{(Eq. 2)}

Carbon Transfer Rate (CTR; gCO₂/L.h) is the amount of CO₂ that is transferred in the medium volume and required by the cell metabolism for a unit of time (Dianursanti, 2012).

CTR = \Delta(y_{CO₂}). α_{CO₂}

\text{(Eq. 3)}

Where, α_{CO₂} = a constant of CO₂ that contains a fixed number of temperature and pressure, airflow superficial velocity; ∆(y_{CO₂}) = the concentration change of CO₂ in and out of the reactor by the incoming CO₂ concentration, multiplied by 100%

\alpha_{CO₂} = \frac{U_p \cdot A \cdot MCO₂ \cdot P}{V_{med} \cdot R \cdot T}

\text{(Eq. 4)}

Where, \(U_p = \) 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 CO₂ (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 CO₂ transfer rate (qCO₂; gCO₂/g.cell.h) is the rate of CO₂ that is transferred in a medium volume due to the activity of biological life within a unit of time.

q_{CO₂} = \frac{\Delta y_{CO₂} \cdot \alpha_{CO₂}}{X}

\text{(Eq. 5)}

Where, \(X = \) cell dry weight per unit volume (g/L).

An approximate formula (CO₂.euH₁₋₈₃N₀₋₁₁P₀₋₁) suggested by Grobbelaar (2004) was used to make an expected estimate of the dry biomass yield. CO₂ utilization efficiency was determined by using the following equation (Ryu et al., 2009):

CO₂ utilization efficiency (%) = 0.57 \times \frac{P \times [(44/12) / V_{CO₂}]}{X} \times 100

\text{(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 CO₂ supplied to the microalgae culture medium (g CO₂/L.h).

**RESULTS AND DISCUSSIONS**

Effect of light intensity, photoperiodism and temperature were discussed in term of growth response and its relation with CO₂ 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 CO₂ 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 CO₂ removal efficiency compare to CO₂ 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 CO₂ by diffusion, and
In this study, the value of the superficial velocity \((U_g)\) is the gas volumetric rate divided by the cross-sectional area. In this study, the value of the superficial velocity was 652.23 dm\(^2\)/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 \(\text{CO}_2\) working well in this growth medium. \(\text{CO}_2\) fixation by microalgae consortium in a vertical bubble column photobioreactor is first marked by a difference in concentration of \(\text{CO}_2\) that input into the reactor and the concentration of \(\text{CO}_2\) coming out of the reactor. The difference in \(\text{CO}_2\) concentration shows that there is a transfer of \(\text{CO}_2\) from the air into the microalgae cultivation media. The rate of \(\text{CO}_2\) fixation by microalgae \(\text{qCO}_2\) is \(\text{CO}_2\) that is transferred to the medium due to microbiological activity in a unit of time. Carbon fixation rate \(\text{qCO}_2\) value can be determined based on the CTR values divided by the dry biomass of microalgae.

Table 1 also shows \(\text{qCO}_2\) value was inversely proportional to the production of dry biomass produced by microalgae. The value of \(\text{qCO}_2\) is a comparison between the amount of \(\text{CO}_2\) transferred to the medium and the amount of biomass produced. The greater the value of dry biomass of microalgae, the smaller the value \(\text{qCO}_2\). It was represented the ability of \(\text{CO}_2\) 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% \(\text{CO}_2\). The increase in \(\text{CO}_2\) 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% \(\text{CO}_2\), 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% \(\text{CO}_2\) significantly.

Ryu et al. (2009) analyzed the impact of \(\text{CO}_2\) 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% \(\text{CO}_2\) and the minimum cell concentration of 1.16 g/L was found at 0.5% \(\text{CO}_2\) mixed in air. They suggested keeping the \(\text{CO}_2\) concentration lower than 5% because higher \(\text{CO}_2\) concentrations might inhibit microalgae growth. The other researchers Chiu et al.
(2009) demonstrated that the CO₂ 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.

Table 2 showed that same thing with biomass dry weight. light dark conditions (24/0) and (16/8) did not show the increase in CO₂ removal efficiency significantly by increasing 2% to 5% CO₂ concentration. However, in the light-dark conditions (16/8) obtained CO₂ removal efficiency 2-fold higher than in dark light conditions (12/12). This suggested that the removal of CO₂ 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).

CO₂ 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 CO₂ into the cell microalgae decreased. In this experiment, CO₂ 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 CO₂ concentrations.

Table 2: Average of CO₂ removal efficiency, carbon transfer rate and carbon fixation rate as a result of variety light photoperiodism.

<table>
<thead>
<tr>
<th>Light photoperiod (light/dark: h)</th>
<th>CO₂ concentration</th>
<th>CO₂ removal efficiency (%)</th>
<th>Carbon transfer rate (gCO₂/L.h)</th>
<th>Carbon fixation rate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/0</td>
<td>2%</td>
<td>33.58</td>
<td>70.72</td>
<td>39.50</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>39.03</td>
<td>81.53</td>
<td>38.39</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>18.05</td>
<td>39.85</td>
<td>36.52</td>
</tr>
<tr>
<td>16/8</td>
<td>2%</td>
<td>36.33</td>
<td>76.35</td>
<td>34.84</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>43.13</td>
<td>90.24</td>
<td>36.52</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>21.03</td>
<td>44.63</td>
<td>34.84</td>
</tr>
<tr>
<td>12/12</td>
<td>2%</td>
<td>20.49</td>
<td>43.46</td>
<td>29.90</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>22.80</td>
<td>48.32</td>
<td>20.23</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>11.02</td>
<td>23.39</td>
<td>29.70</td>
</tr>
</tbody>
</table>

CTR values increased since the beginning of the growth period, when the concentration of CO₂ in the culture medium was below the threshold of saturation, so the CO₂ gas more soluble in the culture medium (Table 2). Trend curves CTR during cultivation is 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 HCO₃⁻ by microalgae. CTR tended to decrease over time at day 10 due to the imbalance between increasing of cells densities and CO₂ biofixation. This condition eventually caused biomass production constant and finally decreased.

Table 2 also shows the values qCO₂ inversely proportional to the value of biomass dry weight during cultivation. The higher growth of microalgae correlated with the smaller value of qCO₂. It was happened because the increased biomass production resulted in less CO₂ available that could be fixed by the cells. Therefore the rate of CO₂ fixation decreased.
The same observation applied to the value of the CTR. The average value of qCO₂ 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 qCO₂ was only 29/h. Differences in qCO₂ values for both conditions was caused by the fact that microalgae did not take carbon source of the CO₂ 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 CO₂ entering the reactor greatly reduced. This phenomenon led to the transfer of CO₂ in the light-dark conditions (12/12) was observed to be smaller than in the light-dark conditions either (24/0) or (16/8).

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) i.e. 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.

The experiment also demonstrated that there were increased in dry weight biomass and CO₂ 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 CO₂ concentrations. CO₂ removal efficiency by microalgae was influenced by several factors, such as CO₂ concentration and flow rate (Ryu et al., 2009), light intensity (Perner-Nochta and Posten, 2007), light/dark photoperiodism (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 CO₂ 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 5:** Carbon transfer rate as a result of variety temperature.

**Figure 6:** Dry weight biomass 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
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.

The optimum temperature for the growth of microalgae was 25-400 °C (Hanagata et al., 1992; Vonschak 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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