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INTEGRATED VERTICAL PHOTOBIOREACTOR SYSTEM FOR CARBON DIOXIDE REMOVAL USING PHOTOTROPHIC MICROALGAE

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Abstract
A vertical photobioreactor containing the microalgae Scenedesmus obliquus is a highly efficient system for converting carbon dioxide (CO_2) into biomass. The use of photobioreactor for CO_2 mitigation has been explored using microalgae as photosynthetic microorganism. The growth rate (μ, h^{-1}) were 0.03; 0.13; 0.20; 0.09 at treatment of 0%, 2%, 5% and 7% pure CO_2 supplied, respectively during 16 days experiment. The maximum dried biomass (gr ml^{-1}) was 1.7 at 2% pure CO_2. The highest CO_2 removal effiency (%) was 34 at 2% pure CO_2 supply. The results showed that the photobioreactor gave a high efficiency of CO_2 removal by phototrophic microalgae culture.

Keywords: vertical photobioreactor, CO_2 removal, microalgae, photosynthetic.

1. Introduction

Microalgae have a large biotechnological potential for producing valuable subtances for the feed, food, CO_2 sequestration, cosmetic and pharmaceutical industries as well as for biotechnological processes. Two distinctive cultural systems have been proposed for CO_2 sequestration with microalgae. One is the open pond system, and the other is the closed photobioreactor system. There is ongoing discussion regarding whether the open pond system or the closed photobioreactor system would be better for CO_2 sequestration [1], [2], [3]. Apparent advantages for utilizing the open pond system are low initial and operational costs. On the other hand, a photobioreactor system has a higher potential productivity due to better environmental control and harvesting efficiency. Compared to open-air systems the PBRs have reduced risk for contamination, more controlled cultivation conditions, better possibility to cause strong turbulent flow, lower CO_2 losses, lower water evaporation, increased volumetric yield, better biomass quality, improved harvesting efficiency and increased illuminated area to volume ratio [4], [5], [6].

Technical systems for the production of phototrophic microorganism are termed photobioreactors (PBR). The design of the technical and technological basis for photo bioreactors is the most important issues for economic success in the field of phototrophic biotechnology. For high-value products in particular, closed systems of photobioreactors seem to be the more promising field for technical development despite very different approaches in design [7]. Several types of photobioreactors have been reported, such as vertical tubular, flat and column photobioreactors. Vertical tubular-type photobioreactors, such as bubble and air-lift photobioreactors, have often been thought to achieve the most efficient mixing and the best volumetric gas

transfer [8].

The CO_2 transfer efficiency is one of the most important parameters for enhancing the CO_2 biofixation rate and algal productivity in a photobioreactor culture system [9]. The carbon dioxide mass transfer capacity of a photobioreactor is determined by the liquid-phase mass transfer coefficient and the specific area available for mass transfer [10]. For the purpose of mass transfer, the most frequently used approach is bubbling CO_2-enriched air into the bottom of the photobioreactor with diffusers [8]. However, the associated drawbacks are a loss of CO_2 to the atmosphere and poor mass transfer rates because of the relatively low inter-facial specific surface area and the low residence times of the gas in the culture [3].

Photosynthesis is the basic process of microalgae-based carbon capture. To study such process a photobioreactor is designed. Photobioreactor is a device that can be used to provide optimum conditions for microalgae to facilitate the process of photosynthesis. In a photobioreactor light, temperature, pH, CO_2 and nutrients can be adjusted [11].

Scenedesmus obliquus ability to absorb CO_2 indicate that it was able to utilize CO_2 as an inorganic carbon source for growth [1], [3], [8]. In this study, the growth of phototrophic *Scenedesmus obliquus* as a group of microorganisms that use CO_2 as a source of energy shown by cell density, dry weight of microalgae biomass and chlorophyll content increasing at exponential phase. Carbon dioxide is the only carbon source for photosynthetic microalgae cultivation. When microalgae absorb CO_2, they not only fix CO_2 but also grow in numbers yielding products such as carotenoids, pigments, proteins, and vitamins that are utilized to make nutraceuticals, pharmaceuticals, animal feed additives and cosmetics [9].

The objectives of this study were : (1) to develop vertical tubular photobioreactor; (2) to study the reflect operating condition and performance of photosynthetic *Scenedesmus obliquus*; and (3) to characterize the growth and CO_2 removal by *Scenedesmus obliquus* at different CO_2 levels.

2. Material and Methods

2.1 Strain Microalgae and Artificial Growth Medium

A Natural phototropic microalga which is *Scenedesmus obliquus* was isolated from Bojongsoang wastewater treatment plant, Bandung, Indonesia. Batch growth experiments were carried out in vertical photobioreactor as closed system were containing of 8 L Provasoli Haematococcus Medium (PHM) injected with various concentration of pure CO_2 gas (0%, 2%, 5%, 7%). Environmental conditions adopted were; 26°C temperature with light intensity of 2500 lux obtained from fluorescent lamp, 8 L min^{-1} of CO_2 flow rate, and 2% of pure gas CO_2 concentration.

2.2 Vertical Photobioreactor System

Vertical bubble column photobioreactor is a vertical column either cylindrical or rectangular filled with growth medium and air was bubbled through a sparged system installed at the bottom. These systems have the highest gas hold up rates which means they have the best mass transfer compared to other systems [12], [13], [14]. CO_2 was injected from the bottom of the column to allow gas mixing with the medium. This is a low cost solution to obtain a high productivity. Sparger attached at the bottom of the reactor to convert the gas into small bubbles [15]. The photobiore- actor with few modifications can be effective for commercial algal biomass multiplication, as well as having implied economic aspects [16]. Another advantage of vertical bubble column photobioreactor that it can be used on a large scale for the culturing of microalgae [17].

In this study, a vertical bubble photobioreactor, with the pore size diameter of sparger 160 μm, was specifically designed to explore the possibility of coupling CO_2 biofixation with microalgal growth. Pure CO_2 was used as feed gas to study the CO_2 removal and growth rate by *Scenedesmus obliquus* in a photobioreactor.

Photobioreactor was made of transparent material, in order to receive adequate lighting to achieve a high-density culture of microalgae. Thus, the efficiency of photosynthesis in an artificial environment is much higher than the natural environment. A schematic diagram of the vertical photobioreactor system is shown in Figure 1.

The vertical photobioreactor was made of

glass with a capacity of 10 L containing 8 L of constructed consortium culture (A). To achieve the objectives of this study, the photobioreactor was equipped with a source of gas from CO_2 gas cylinder (B) and aerator (C), biomass sampling port, gas outlet, and a port for the pH controller (G). Provisions were also made for the gas flowrate meter (D), CO_2output sampling (E), silica gel tube (I), manometer (J), the photobioreactorbase is equipped with a drain hole for the gas input and expenditure biomass (F), fluorescent lamp as the source artificial light (K) and pump (M). An outlet was provided in order to collect the samples for pH, biomass sample and CO_2 concentration measurements. A tubing intercept was provided between the flowrate meter and the inlet for inlet gas measurements, whereas the outlet gas was measured directly near the outlet provided for collecting the sample. The vertical photobioreactor was run in a continuous light mode day and night, with a constant supply of 0%, 2%, 5%, 7% pure CO_2 gas, respectivelly. Photobioreactor and all the accessories are assembled in such a way in a box iron frame covered by the board on all four sides, and fitted wheels to be easily moved.

The inflow pure CO_2 gas was injected into the culture with various concentration 0%, 2%, 5% and 7%, respectively. Three probes for dissolved CO_2, dissolved oxygen (DO) and pH were installed at the top of the photobioreactor. The dissolved carbon dioxide, pH and DO of the algal culture were monitored online and recorded every minute by the controlling system. The choice of 10 L as photobioreactor volume was based on the following reasons, i.e : 1) meet the needs of each sampling volume during the study period and 2) pay attention to the cost of making an artificial medium.

Photobioreactor hydrodynamics aspects include: the dimensions of the tube are a total height of tube 80 cm, diameter 15 cm, height of liquid (growth medium) 65 cm; hose with a diameter of 15 mm for the flow of CO_2 and CO_2 gas cylinder from the aerator, CO_2 input into the holes located at the base of the reactor; sparger placed at the bottom of a vertical tube, precisely on the surface of the vent gas input (D), has an outside diameter of 25 mm, 21 mm in diameter and 100-160 µm pore diameter. Since the bubble have a small size, it become light and have a large surface. So, the bubble is easily broken to be well distributed along the reactor, from bottom to surface, instead of retained in the bottom of the reactor. The gas flow rate 8 L.min^{-1} was maintained at this level in order to keep the culture suspended in the medium. Position 4 TL lamps were uniformly positioned outside the photobioreactor so that they can be adjusted to obtain a light intensity of 2500 lux. The reactor is equipped with a coil on the outside of the tube to facilitate research on the impact of certain temperature stable during the study period.

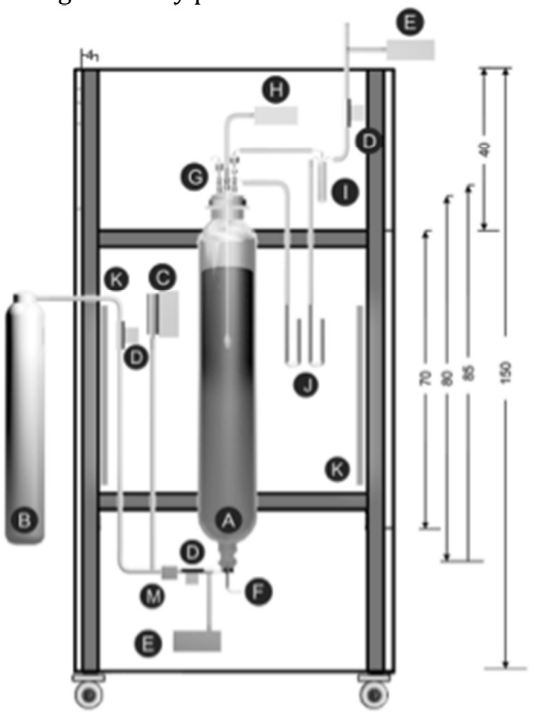

Figure 1: Overview of vertical photobioreactor (not to scale).

2.3 Measurement of light intensity

A light sensor (TES1332A, Taiwan) was used to measure the light intensity on the surface of the bioreactor. The average light intensity was calculated by taking the weighted average of 60 measurements, which are located at the three horizontal planes uniformly located along the vertical axis of the reactor, and 20 evenly distributed measuring points along the radial axis for each horizontal plane height of the reactor [9].

2.4 Measurement of biomass concentration

The biomass concentration (g.L^{-1}) was

measured by analyzing the optical density of the cell suspension at an absorbance wavelength of 665 nm using a spectrophotometer (thermo scientific type spectronic + 20, Germany). A calibrated straight line was obtained previously by plotting the A665 dry cell weight ($g.L^{-1}$).

2.5 Measurement of dry mass (suspended solids)

The dry mass of all the microalgae species was determined on triplicate culture samples. Suspended solids tests were conducted on microalgae culture, which was diluted to approximately 8 different optical density values. Equal volumes of the microalgae samples were filtered through a 47 mm Whatman® (Maidstone, England) GF/C glass-micro fiber filter, which were previously ashed in an oven at 550°C for one hour and weighed for their initial weight. A suction pump was used to filter the samples and after filtration the filters were dried in a WTB Binder Laboratory oven made in Germany at 105-110°C for approximately 24 hours. The filter was taken and cooled to room temperature in a desiccator and was weighed to get the final weight [4]. The difference in the weights of the filter, after filtration and before filtration, divided by the sample volume filtered gave the dry mass. A plot of the optical density at 665 nm and dry mass in mg/l was developed.

2.6 Measurement of growth rate of the microalgae

A regression equation of the cell density and dry weight per liter of culture was obtained by a spectrophotometric method. Specific growth rate (μ) of microalgae is calculated by the following formula :

$$\mu = \frac{N_t - N_0}{t - t_0} \times \frac{1}{N_t} \qquad (1)$$

Where, μ = specific growth rate (cell biomass /ml/unit time); N_n = Initial cell culture density at time t_0 (cell/ml); N_t = Culture density at time t (cell/ml); $t\text{-}t_0$ =time interval (day). Cell density (N) is obtained from calibration curve OD 665 vs X with a spectrophotometer.

2.7 Analysis of CO_2 and O_2 concentration

The CO_2 and O_2 concentration in the influent gas and effluent gas were measured 5 times per day by Portable Combination Gas Detector RIKEN Model RX-515.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 a day using the Portable Carbon Dioxide Analyzer Oxyguard to know the solubility of CO_2 in the culture medium. From this measurement, the capability (efficacy) of the photobioreactor microalgae cultivation system as a CO_2capture storage agent can be determinated.

2.8 CO_2 removal efficiency

The CO_2 biofixation rate and the CO_2removal efficiency were calculated by the following equations:

$$CO_2\ biofixation\ rate = \frac{X_{max} - X_o}{t} \times \frac{C}{12} \times 44 \qquad (2)$$

$$CO_2\ removal\ efficiency = \frac{Y_{in} - Y_{out}}{Y_{in}} \times 100\% \qquad (3)$$

where, X_{max} and X_0 are the maximum cell concentration and the initial inoculated cell concentration (mg/L); t is the time required to reach the maximum cell concentration (d); C is the carbon content of dried biomass analyzed by the elemental analyzer; 44 is the molecular weight of CO_2, and Y_{in} and Y_{out} are the CO_2 molar fractions in the inlet and outlet gas phases, respectively.

3. Result and discussion

3.1 Cell density

Figure 2 shows that cell densities in all the cultures increased up to day 8 for all pure CO2 concentration input. On supply 0% CO_2, cell density decreased in 1-2 day culture period, but the next day the cell density increased although this increment was not as high as the culture with 2%, 5% and 7% CO_2, respectively. Treatment with 5% CO_2 produced a higher cell density compared to treatment with 0%, 2% and 7% CO_2. Each of the known photobioreactor types has some advantages as well as disadvantages. However, regardless of reactor design chosen, the same technical requirements must be met in order to achieve maximimum growth of microalgae.

According to Kunjapur and Elridge [7], the ultimate goal of PBR design is to maximize the specific growth rate (μ) defined as "an increase in the mass of cells in culture per unit time per unit mass of cells." Specific growth rate (μ, h^{-1}) of *Scenedesmus obliquus* in the exponential phase ranging from the fastest to the slowest for 2% pure CO_2 supply, 5% CO_2 and 7% CO_2 and 0% were 0.2791; 0, 2651; 0.1162 and 0.1038 respectively.

3.2 Dry biomass

Apart from observing the cell density, the growth response of S*cenedesmus obliquus* can be measured by weighing the dry biomass produced. Treatment with 0% CO_2 treatment did not produce any significant increase in biomass dry weight throughout the study period in all the cultures. There were fluctuations in the dry weight of *Scenedesmus obliquus* cultures. However, a decrease in the dry weight biomass was observed around day 8 or 9.

Treatment with 5% CO_2 input provides the highest dry biomass compared with treatment 2% and 7% (Figure 3). Maximum dry biomass average (gr/100 mL) at treatment supply 0%, 2%, 5%, 7% pure CO_2 were at 0.22; 1.69; 1.23 and 0.82 respectively. As photosynthetic organisms, microalgae are well adapted to capture ambient CO_2. Growing algae that capture ambient CO_2 will remove carbon dioxide and sequester it in the form of biomass [18].

3.3 Carbon dioxide removal efficiency

The amount of CO_2 absorbed (concentration in %) by microalgae obtained from the difference between the pure CO_2 injected into the photobioreactor with a CO_2 output out of the photobioreactor, taking into account the CO_2 dissolved in solution or culture growth medium. In this study, the CO_2 dissolved in the medium is very small and relatively constant through out the study period. *Scenedesmus obliquus* can absorb 2% to 7% pure CO_2.

All cultures showed efficiencies above 10% for treatments with 2%, 5%, 7% CO_2 (Figure 4). Although microalgae are considered to be relatively efficient for capturing solar energy for the production of organic compounds via photosynthetic process, the photosynthetic efficiency of microalgae for the conversion of solar energy is typically below 20% [19], [20]. On the other hand, increasing the density of cultures decreases photon availability to individual cells, which reduces specific growth rate of cells. Therefore, the poor penetration of light could be the most significant limiting factor in microalgal cultivation. Chiu *et al*. [21] observed that the maximum efficiency of CO_2 removal was 63% (with 10% CO_2 in the aeration gas).

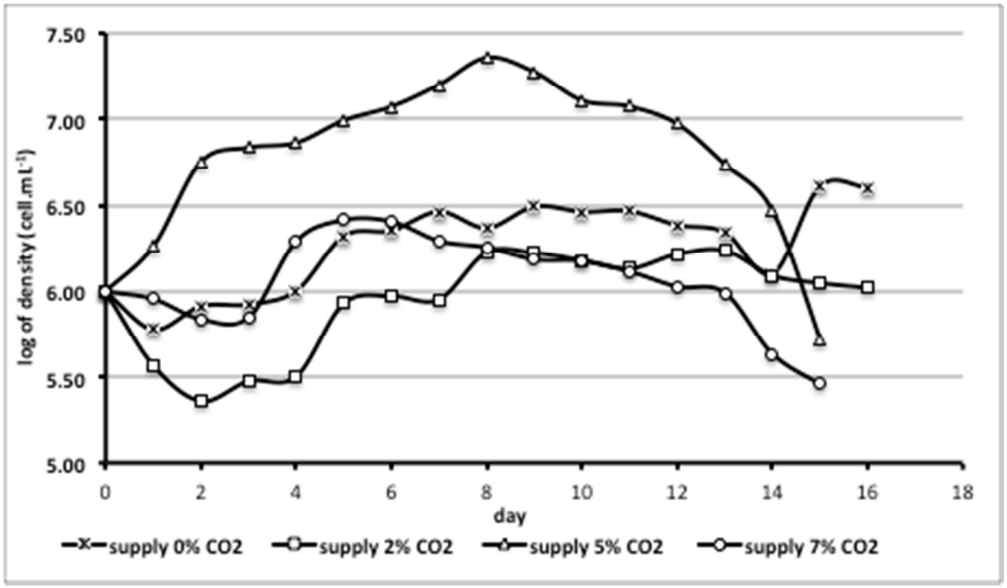

Figure 2 : Cell density of Scenedesmus obliquus (10^6 cell.ml^{-1}) in each treatment of pure CO_2 supplies, room temperature (26 ±1^{0}C), pH 7.14 ± 0.31, flow rate of CO_2 8 L.m^{-1}, light intensity 2500 lux, photoperiodism light/dark (24 hours/0 hours)

3.4 Carbon dioxide fixation rate

According to an elemental analiysis in the *Scenedesmus obliquus* cell at the end of cultivation, 1 g of biomass contained approximately 0.563 g carbon in the study. The value was used to estimate the CO_2biofixation rate ($mg.L^{-1}.day^{-1}$). The CO_2 biofixation rate was calculated according to Equation 2, and the results are presented in Table 1. Figure 5 shows the CO_2 biofixation rate profiles of the *Scenedesmus obliquus* under different level of CO_2 concentration (%). Although the culture that was supplied with 7% CO_2 on day 3 had showed the highest of CO_2 fixation rate but the highest average of CO_2 fixation occurred in the culture that supplied with 2% CO_2 (-8.7 $mg.L^{-1}.day^{-1}$). The lowest biomass concentration and CO_2 biofixation rate were observed when the culture didn't supply with CO_2.

4. Conclusion

Microalgae biofixation of CO_2 in photobioreactors has recently gained renewed interest as a promising strategy for CO_2 mitigation. Using a microalgal photobioreactor as a CO_2 mitigation system is a practical approach to the problem of CO_2 emission from waste gas. A vertical photobioreactor containing *Scenedesmus obliquus* is a highly efficient system for converting carbon dioxide (CO_2) into biomass. *Scenedesmus obliquus* were cultivated in a vertical photobioreactor input of 2%, 5%, and 7%, pure CO_2, all of them can grow well, which was observed by cell density, dried biomass and specific growth rate (μ).The highest CO_2 removal efficiency (%) was 34 at 2% pure CO_2. The results showed that the photobioreactor worked properly for CO_2 removal by phototrophic microalgae culture. CO_2 removal efficiency, CO_2 biofixation rate and specific growth rate will be improved in future studies, to examine the effects of temperature, light intensity and hydrodynamic aspects of photobioreactor.

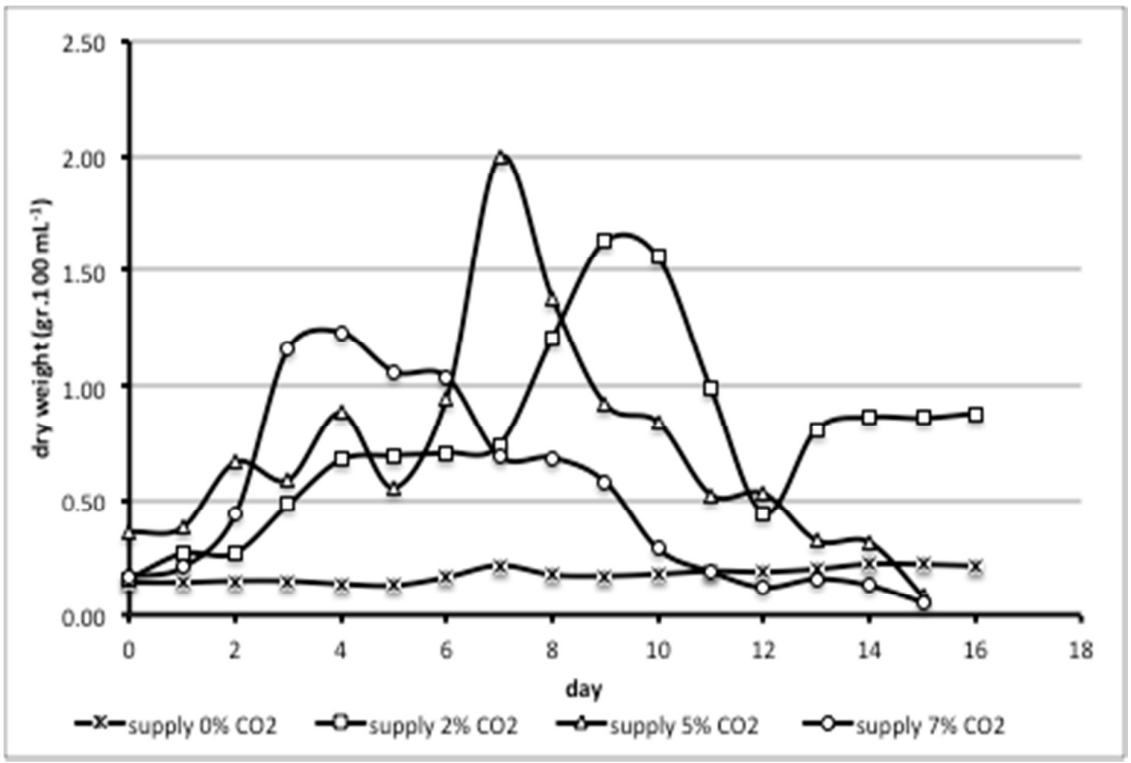

Figure 3: Dry biomass of Scenedesmus obliquus in each treatment of pure CO_2 supplies, room temperature (26 $\pm 1^0$C), pH 7.14 $\pm$ 0.31, flow rate of CO_2 8L.m^{-1}, light intensity 2500 lux, photoperiodism light / dark (24 hours/0 hours)

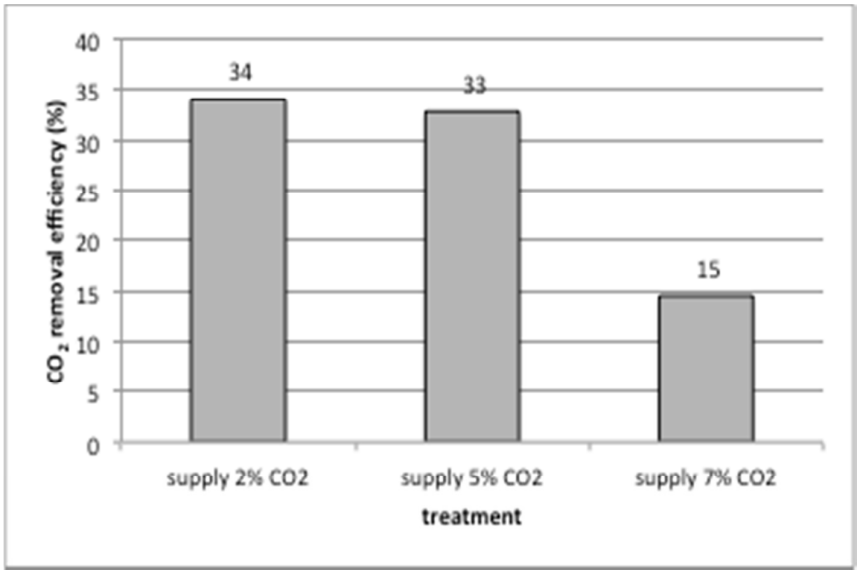

Figure 4: CO_2 removal efficiency of Scenedesmus obliquus in each treatment of pure CO_2 supplies, room temperature (26 ±1⁰C), pH 7.14 ± 0.31, flow rate of CO_2 8 L.m^{-1}, light intensity 2500 lux, photoperiodism light/dark (24 hours/0 hours)

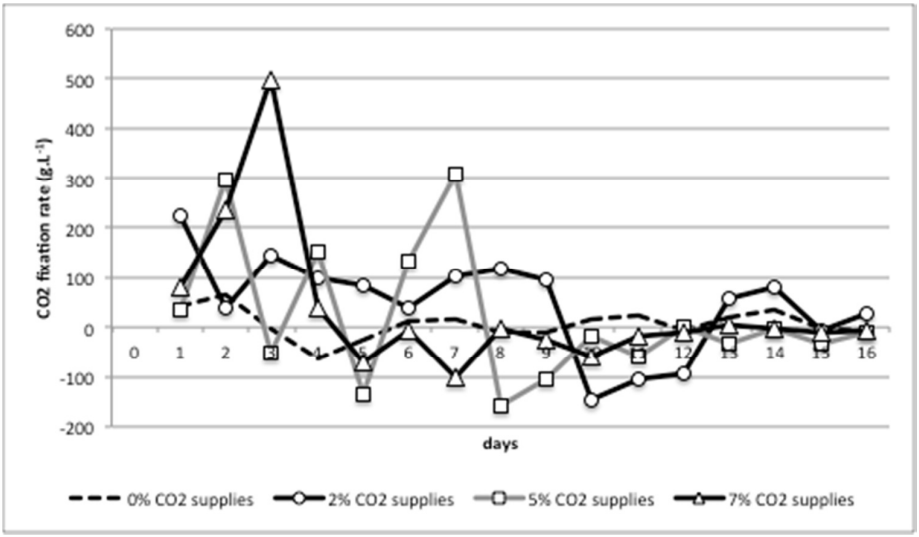

Figure 5: CO_2fixation rate of Scenedesmus obliquus in each treatment of pure CO_2 supplies, room temperature (26 ±1⁰C), pH 7.14 ± 0.31, flow rate of CO_2 8 L.m^{-1}, light intensity 2500 lux, photoperiodism light/dark (24 hours/0 hours)

Table 1. Average of CO_2 fixation rate (mg.L^{-1}.d^{-1}) of Scenedesmus obliquus in each treatment of pure CO_2 supplies

Pure CO_2 supplies (%)	Maximum dry weight biomass (g.100mL^{-1})	Biofixation rate (g.L^{-1}.d^{-1})
0%	0.22	-1.9
2%	1.69	-8.7
5%	1.23	-18.4
7%	0.82	-25.9

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