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Biotechnology Carbon Capture and Storage (CCS) by Mix-culture Green Microalgae to Enhancing Carbon Uptake Rate and Carbon Dioxide Removal Efficiency with Variation Aeration Rates in Closed System Photobioreactor

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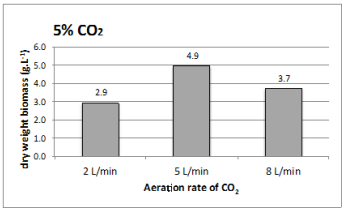
Graphical abstract



Abstract

Carbon dioxide (CO_2) sequestration by green microalgae is receiving increased attention in alleviating the impact of increasing CO_2 in the atmosphere. The goal of this study was to explore the capacity of mixed culture green microalgae *Chlorella* sp, *Scenedesmus obliquus*, and *Ankistrodesmus* sp. as carbon capture and storage agent to enhance CO_2 uptake rate and CO_2 removal efficiency which was observed at elevated CO_2 aeration rates of 2, 5, and 8 L min^{-1} supplied to vertical photobioreactor continuously in batch system culture. The operation condition of this research were 6.5-7.5 pH, temperature of 30^0C, light intensity of 4000 lux with 16 hours light period and 8 hours dark period, and high pure CO_2 elevated level of 5 to 18 (concentration in %; v/v in the aeration gas) as inorganic carbon. The maximum CO_2 removal efficiency of the mix culture was 59.80% when the biomass was obtained at 4.90 gL^{-1} and CO_2 flow rate ($Lmin^{-1}$) of 5 vvm in a vertical photobioreactor. The value of CO_2 removal efficiency improved by almost 200% and 120% as compared to that in the low and high aeration rate (2 $Lmin^{-1}$ and 8 $Lmin^{-1}$) respectively. The CO_2 up take rate of a mixed culture reach 979.62 mg carbon $L^{-1}day^{-1}$, which was enhancing by 3-fold in high aeration rate (8 $Lmin^{-1}$). The results showed that the CO_2 removal efficiency and carbon uptake rate was related to biomass concentration and aeration rate of CO_2 supplied.

Keywords : Carbon uptake rate; carbon dioxide removal efficiency; photobioreactor; microalgae

1.0 INTRODUCTION

Carbon dioxide is usually emitted freely from industrial processes in an uncontrolled way. CO_2 concentration in the troposphere is getting serious attention as CO_2 is categorized as greenhouse gas that is believed to be the cause of global warming. Impacts of greenhouse gases are becoming more apparent mainly due to the increase of the earth's surface temperature [1-3]. Biological carbon capture and storage (CCS) technologies can be used to mitigate carbon emissions that would otherwise be released to the atmosphere. Research studies that utilized the potential of microalgae as CCS agent have been carried out in various countries, particularly in efforts towards adaptation and selection of microalgae species tolerant to high CO_2 concentrations and high CO_2 absorption rate. Most of the flue gases produced by most concentration make it advantageous for the microalgae to be the best candidate for creating a sustainable carbon sink. Since microalgae are producers, they have the ability to continuously undertake photosynthesis. One of the primary requirements for photosynthesis is atmospheric CO_2. Growing microalgae that captures ambient CO_2 will remove carbon dioxide and sequester it in the form of biomass [4].

When CO_2 is injected in a culture, a concentration gradient builds up as it is consumed by cells and/or lost to the atmosphere. According to the two-film theory, mass transfer of CO_2 from the gas phase to the cell phase occurs through sequential stages [5-6]. The mass transfer of carbon dioxide from air into the growth media can be growth-limiting in dense microalgae cultures and its process photosynthesis. Milne *et al.* stated that the transfer of CO_2 from a gas to a liquid depends on many parameters [7]. Physical parameters such as gas aeration rate, CO_2 partial pressure, bubble diameter and lifetime can have large influences on the rate of transfer. Other studies have shown higher values of overall mass transfer coefficient are obtained at higher gas velocities [8]. An increase in superficial gas velocity causes an increase in gas holdup, which increases the interfacial area. There is an increase in interfacial area because higher gas velocity leads to higher momentum exchange between phases. As a result, bubbles break at a higher efficiency into smaller bubbles and the interfacial area becomes bigger.

The aim of the study was to observe the impact of different aeration rates of air-rich CO_2 to enhanced CO_2 removal efficiency and carbon uptake rate by mixed-culture green microalgae cultivated in vertical bubble photobioreactor.

2.0 MATERIAL AND METHODS

2.1 Mix-culture Microalgae and Arificial Growth Medium

The mix-culture green microalgae consisting of *Chlorella* sp., *Scenedesmus obliquus* and *Ankistrodesmus* sp. was originally isolated from the Bojong Soang wastewater treatment plant, Bandung, Indonesia. The microalgal was screened, and then a potential candidate was selected for Microbial Carbon Capture and Storage (MCCS) agent [9]. The microalgal cells were cultured in PHM (Phovasoli Haematococcus Media) artificial growth media [10].

2.2 Cultivation Microalgae in Vertical Photobioreactor and Experimental Condition

Vertical photobioreactor made of glass with a capacity of 10 L containing by 8 L PHM growth medium and an initial cell density of 10^6 cell.ml^{-1}. The pure CO_2 gas supplied from the bottom of the photobioreactor with 5%, 10%, 15%, and 18% pure CO_2 level at different CO_2 aeration rate of 2.0, 5.0, and 8.0 L.min^{-1}, respectively, and temperature was adjusted at 30^0C $\pm$ 1. CO_2 was injected from the bottom of the column to allow gas mixing with the medium. Sparger was attached at the bottom of the photobioreactor to convert the gas into small bubbles. Air is bubbled at the bottom. Microbubble sparging allows thorough mixing, CO_2 mass transfer and also removes O_2 produced during photo-synthesis. It was a strategy that provides good overall mixing, sufficient supply of CO_2, and efficient removal of O_2. Position 4 TL lamps uniformly placed outside the photobioreactor can be adjusted to obtain a light intensity of 4000 lux and light periods (light/dark; hour) of 16/8.

2.3 Measurement of Biomass Concentration and Growth rate of the Mix-culture Microalgae

Dry weight cell biomass of microalgae was obtained by evaporating the liquid in the cell culture. A total of 100 mL culture tube inserted into centrifuges, and then centrifuged at 3500 rpm for 10 minutes [11]. 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 105^0C for one night to get a constant weight (y), and then stored in a desiccator for 30 minutes before re-weighed. Biomass (dry weight) to calculated by the formula: dry weight (X; mg) = y (mg) - x (mg). Specific growth rate (μ; d^{-1}) was calculated as follows [12]:

$$\mu = \frac{1}{X}.\frac{dX}{dt} \quad (1)$$

2.4 Measurement of CO_2 Concentration and Determination of CO_2 Removal Efficiency

The CO_2 concentration in the influent gas and effluent gas was measured by Portable Combination Gas Detector RIKEN Model RX-515. Efficiency of CO_2 removal can be calculated by the following formula:

$$\frac{\text{Influent of } CO_2 - \text{Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\% \quad (2)$$

An approximate formula ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) was used to make an expected estimate of the dry biomass yield [13] and carbon uptake rate was determined by using the following equation [14]:

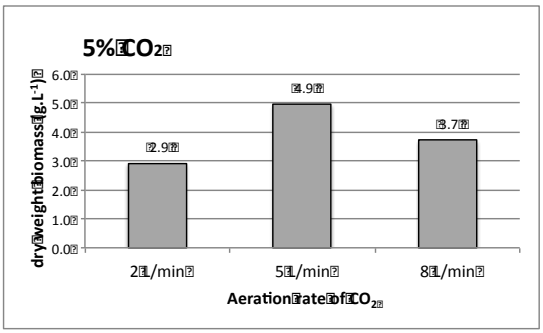
$$\text{Carbon uptake rate} = C \times P \quad (3)$$

Where, C is the carbon content of the dry weight cell (g carbon.g biomass^{-1}), P is the productivity (g biomass.L^{-1}d^{-1}). Results of elemental analysis in our study showed that the carbon content in the mix culture was 67.56%.

3.0 RESULTS AND DISCUSSION

3.1 Dry Weight of Biomass as Growth Response

Our previous study obtained the highest dry weight of biomass occurred from the culture which was supplied continuously with 5% (v/v) pure CO_2 [15]. Thus, the study of the impact of CO_2 aeration rates started with supplied 5% (v/v) pure CO_2.



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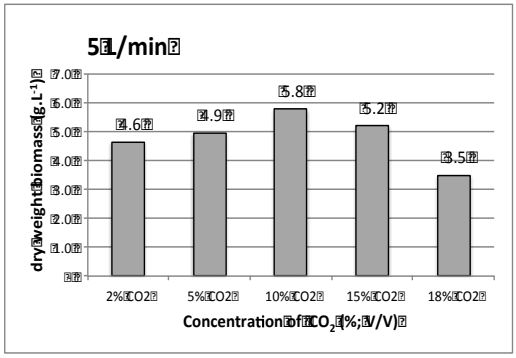


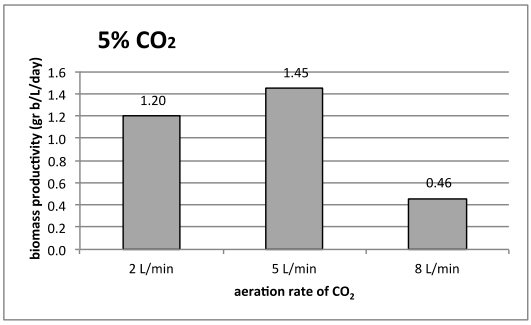
b)

Figure 1 Dry weight biomass (a) with variation aeration rate of CO_2 and 5% concentration of CO_2, (b) with variation concentration of CO_2 (in %; v/v), CO_2 aeration rate of 5 L/min, all were supplied continuously

An increase in concentration of the CO_2 regardless of the flow rate resulted in a decrease in pH [16]. However, this study showed that aeration rate has a great influence on both growth and dry biomass yield because the growth medium has a weak buffering capacity. The pH drastically decreases when high level of CO_2 gas was supplied. It is possible that the low pH observed when pure CO_2 was used could have been the reason for the reduced growth rates. When dissolving in water, CO_2 equilibrates

into CO_2 (aq), HCO_3^- (aq),and CO_3^-(aq).This lowers the pH, whereas at a pH of 6 and lower, CO_2 (aq) is dominant. At a pH of 6–9, HCO_3^-(aq) becomes more pronounced, and at a pH of 9 and above, CO_3^{2-} becomes predominant [16].

Another study showed that *Scenedemus* sp. and *Chlorella* sp. had a long lag phase in very high concentrations of CO_2 [17]. They further suggested that the dry weight biomass was not affected by variation in the flow rates of air containing elevated CO_2. This was the result of their use of sea water, which has a strong buffering capacity. In contrary, our experiment use of fresh water containing macro and micro nutrient as artificial growth media, thus the dry weight biomass was affected by variation in the flow rates of air containing elevated CO_2 (Figure 1(b)).



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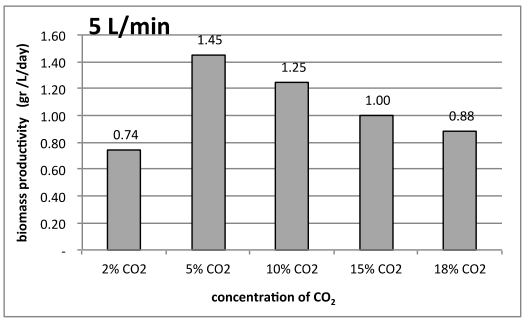


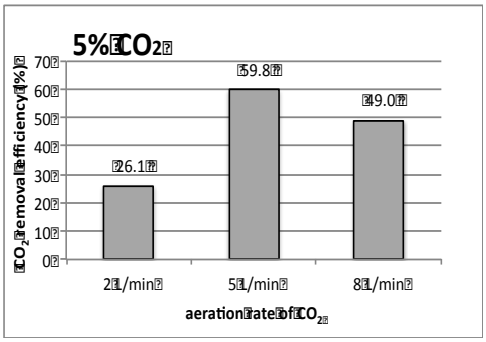
b)

Figure 2 Biomass productivities (a) with variation aeration rate of CO_2 and 5% concentration of CO_2, (b) with variation concentration of CO_2 (in %; v/v), CO_2 aeration rate of 5 $Lmin^{-1}$, all were supplied continuously

Figure 2(a) shows that biomass productivity in culture that supplied with 5% CO_2, aeration rate of 2 $Lmin^{-1}$ and 5 $Lmin^{-1}$ were not different significantly. The highest biomass productivity (1.45 $g.L^{-1}$), was found at aeration rate of 5 $Lmin^{-1}$. It means the biomass productivity increased 3-fold highest compare to cultures in aeration rate of 8 $Lmin^{-1}$. However, Figure 2(b) shows the biomass productivities in culture that supplied more than 5% CO_2 were getting decrease, probably because increasing of CO_2 level become toxicity for growing microalgae.

3.2 Carbon Dioxide Removal Efficiency

CO_2 removal in a vertical bubble column photobioreactor is first marked by a difference in concentration of CO_2 then input into the reactor and the concentration of CO_2 coming out of the reactor. Difference in CO_2 concentration shows that there is a process of removing CO_2 from the air into the microalgae cultivation media (Equation 2).



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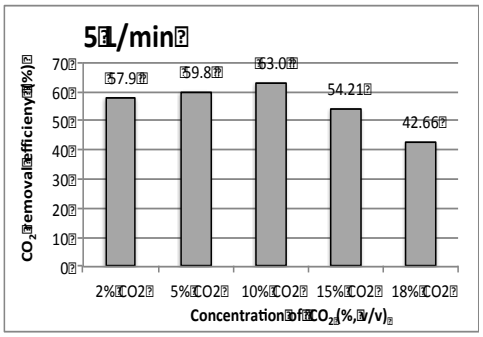


b)

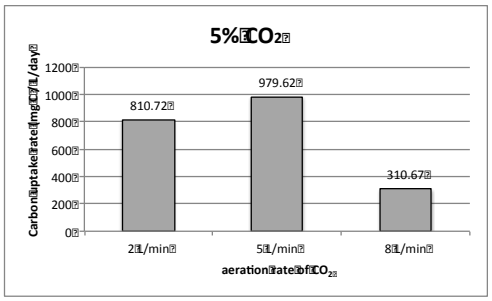
Figure 3 CO_2 removal efficiency (a) with variation aeration rate of CO_2 and 5% concentration of CO_2, (b) with variation concentration of CO_2 (in %; v/v), CO_2 aeration rate of 5 $Lmin^{-1}$, all were supplied continuously

At the same level of CO_2 (5%), although dry weight biomass in aeration rate of 2 $Lmin^{-1}$ was not increasing significantly as compared with 5 $Lmin^{-1}$, however the CO_2 removal efficiency in aeration rate of 5 $Lmin^{-1}$ was increasing at 2-fold than aeration rate of 2 $Lmin^{-1}$ (Table 1, Figure 3(b)) which shows CO_2 of 8 $Lmin^{-1}$ was lower than at 5 $Lmin^{-1}$. The CO_2 removal efficiency decreased with increasing gas flow [18].

The reason for this decrease can be explained by the increased gas hold up and turbulence caused in the system because of higher gas flow rates. Under such condition, excessive presence of extremely small gas bubbles were formed which did not participate in the mass transfer of the gas to the liquid phase. Therefore, when the system was highly turbulent there was more gas hold up that forces more CO_2 to leave the photobioreactor system. The highest CO_2 removal efficiency was obtained from the culture which was supplied with CO_2 aeration rate of 5 $Lmin^{-1}$ and 10% CO_2 (Figure 3(b)).

3.3 Carbon Uptake Rate

All algae could take up CO_2 by diffusion, and many had active carbon uptake systems which could take up bicarbonate (HCO_3^-). However, microalgae could not take up the CO_3^{2-} ions [19].



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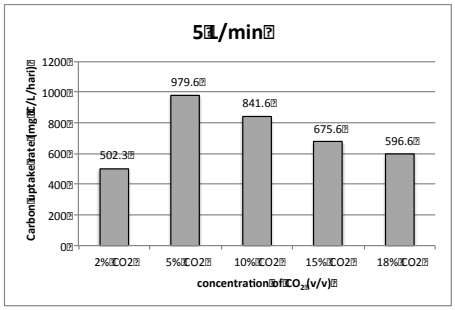

b)

Figure 4 Carbon uptake rate (a) with variation aeration rate of CO_2 and 5% concentration of CO_2, (b) with variation concentration of CO_2 (in %; v/v), CO_2 aeration rate of 5 L/min, all were supplied continuously

Studies have been undertaken to compare low aeration rate (2 $Lmin^{-1}$) and high aeration rate (8 $Lmin^{-1}$) with the same high concentration (%, v/v) of CO_2, and it was observed that carbon uptake rate (mg $C.L^{-1}d^{-1}$) were higher in low aeration rate than the high aeration rate, which the values were 810.72 and 310.67, respectively (Figure 4(a)). The highest value of carbon uptake rate of 979.62 mg $C.L^{-1}d^{-1}$ was recorded from the culture with aeration rate of 5 $Lmin^{-1}$. In the next experiment (Figure 4(b)) with higher level of CO_2 and the same aeration rate (5 $Lmin^{-1}$) showed that carbon uptake rate getting decreased in culture that supplied with concentration of CO_2 higher than 5% (v/v).

Many researchers describe that efficiently capturing carbon dioxide and carbon uptake rate from an elevated CO_2 source depends on many factors [23-26], but one of the most limiting factors at present is the ability of the microalgae to capture and fix carbon at a proper concentration to avoid acidification of the medium and crash of the culture, all of which could inhibit the growth of microalgae. It has proven that growth rate (μ) in a culture that was supplied with 10% CO_2 was lower than the culture that was supplied with 5% CO_2, i.e. the values of growth rate were 0.43 and 0.32 respectively (Table 1).

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

Table 1 Comparison of growth response, carbon dioxide removal efficiency, and carbon uptake rate under variation of carbon dioxide aeration rate

	CO_2 Aeration rate				
	2Lmin^{-1}	**8Lmin^{-1}**	**5Lmin^{-1}**	**5Lmin^{-1}**	**Literature**
CO_2 removal efficiency (%; v/v)	**26.10**	**49.00**	**59.80**	**63.10**	▪ 65 [20]; 85 [21] ▪ 45 [22]; 52 [23]
Concentration CO_2 max that gives highest CO_2 removal efficiency (%)	**5%**	**5%**	**5%**	**10%**	▪ 10% [20]; ▪ 10% [21] ▪ 10% [22]; ▪ 10% [23]
Dry weight biomass (g/L)	**2.9**	**3.7**	**4.9**	**5.8**	▪ 2.046 [23]
Growth rate (μ; per day)	**0.38**	**0.18**	**0.43**	**0.32**	▪ ND [20]; ▪ ND [21] ▪ 0.252 [22]; ▪ 011 [23]
Biomass productifity (P; g biomass $L^{-1}day^{-1}$)	**1.20**	**0.46**	**1.45**	**1.25**	▪ 0.94 [20]; ▪ 0.632 [21]; ▪ 0.3818 [22] ▪ 0.610 [23]
Carbon consumption rate = Carbon uptake rate (mg Carbon $L^{-1}day^{-1}$)	**810.72**	**310.67**	**979.62**	**841.6**	▪ 1316 [20] ▪ 1367[21] ▪ 717.8 [22] ▪ 1147 [23]

We assumed that CO_2/O_2-balance is also a prime factor in achieving a higher carbon uptake rate. For this reason, other researchers assume that the carboxyl enzyme, Rubisco, utilizes CO_2 via the Calvin cycle to turn the carbon source into bio-energy, an excess of oxygen may become a problem in the algal culture, not only because it can limit the photosynthesis rate (photorespiration) as well as carbon uptake rate, but also because oxygen radicals may have toxic effects and cause cell membrane damage [27].

■4.0 CONCLUSION

This study showed that aeration rate has a great influence on both removal CO_2 efficiency and carbon uptake rate. These results imply that mix-culture green microalgae can tolerate high concentration of CO_2 at aeration rates of 2 $Lmin^{-1}$ to 5 $Lmin^{-1}$. Therefore, when designing CO_2 sequestration systems for microalgae, it should ensure the flow rate is maintained below 5 $L.min^{-1}$ levels, to allow maximum CO_2 mass transfer into microalgal biomass.

Acknowledgement

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