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CARBON DIOXIDE FIXATION BY CO-CULTURE GREEN MICROALGAE THROUGH OVERALL VOLUMETRIC MASS TRANSFER COEFFICIENT ($K_a$) OF CARBON DIOXIDE IN CLOSED SYSTEM

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Key words: CO₂ removal, Biomitigation, CO₂ fixation, Volumetric mass transfer coefficient, Co-culture.

Abstract—Culture conditions are very important to CO₂ bio-fixation but its implementation is limited by the poor mass transfer efficiency. The three green microalgae, i.e. Chlorella, Scenedesmus obliquus, and Ankistradenum sp were cultured as co-culture in a vertical bubble photobioreactor as closed system, with 160 µm size diameter of pore membrane sparger to explore the possibility of coupling CO₂ biofixation affected the carbon mass transfer process. In this study, overall volumetric mass transfer coefficient ($K_a$) of CO₂ were developed with operational culture conditions at temperatures ($°C$) of 30, light intensities (lux) of 4000 and photoperiodism (light/dark; hour) of 16/8, with various high concentration (%) CO₂ supplied from the bottom of photobioreactor were 0, 2, 5, 7. The result showed that the highest $K_a$ value (h⁻¹) was 0.1419 5% pure CO₂ supplied, gas flow rate 8 L/min during 12 days experiment. While at this condition, the maximum dried biomass (g/mL) was 2.7 ($µ$ = 0.38) at 5% pure CO₂. It could be concluded that CO₂ removal efficiency (%) affected by the $K_a$ value, whereas the highest CO₂ removal efficiency (%) of 49.02 occurred when the Kla value was highest.

INTRODUCTION

There are three cases of mass transfer: liquid-solid mass transfer, liquid-liquid mass transfer and gas-liquid mass transfer. In this work, gas-liquid mass transfer will be discussed to analyze the mass transfer coefficient of carbon dioxide in water. Carbon dioxide is usually the main carbon source in the photosynthetic cultivation of microalgae and can be transferred continually or intermittently from the gas phase to the liquid phase of the culture medium (Grima et al., 1999). (When dissolving in water, CO₂ equilibrates into CO₂(aq), HCO₃⁻(aq), and CO₃²⁻(aq). This lowers the pH, whereas at a pH of 6 and lower, CO₂(aq) is dominant. At a pH of 6–9, HCO₃⁻(aq) becomes more pronounced, and at a pH of 9 and above, CO₃²⁻ becomes predominant (Ota et al., 2009). The critical concentration of CO₂ necessary for optimal growth of co-culture microalgae cannot be stated in general, as it strongly depends on the delivery system implemented in the culture vessel. Becker (1995) described that since transfer of CO₂ occurs through the interface between the gaseous mixture and the liquid medium culture, two main processes to increase such an interface area can be devised: (i) passive mode, where extensive gas/culture interface areas are used and gas diffuses into the culture; and (ii) active mode, where use of an extra apparatus for aeration either by injecting the gas into the medium or spraying the medium into the gas forces expansion of the contact area between gas and culture.

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Kazim (2012) suggested that the process of carbon dioxide transfer from the gaseous phase to the liquid phase is dependent on the overall mass transfer coefficient of carbon dioxide. The exact mass transfer coefficient values will help determine the transfer rate of gaseous carbon dioxide to a liquid phase, and the optimal amount of the gas supply needed to increase its consumption by microalgae for maximum possible growth. Singh and Majumder (2010) reported superficial gas velocity is one of several factors which affect the mass transfer coefficient of carbon dioxide.

Carbon dioxide is soluble in water and its solubility has been reported as being higher than other gases such as nitrogen, oxygen, argon, neon, krypton and Helium. In fact, the solubility of carbon dioxide is 26 times higher than oxygen (Kordac and Linek, 2008). Due to carbon dioxide’s high solubility in water, cultivation of microalgae is required optimal K_{L_a}(CO_2). K_{L_a} values demonstrated CO_2 mass transfer process for better microalgae culture. However, not always the high value of K_{L_a}(CO_2) is suitable for the growth of microalgae culture. K_{L_a} values (CO_2) that is too high may cause the shear stress on the microalgae. This phenomenon may inhibit the growth of microalgae, and should be avoided. The purpose of this study was to determine the rate of CO_2 absorption by microalgae, measuring of the mass transfer coefficient (K_{L_a}) and its relation to cell growth and CO_2 removal.

**MATERIALS AND METHODS**

**Cultivation and growth condition in vertical photobioreactor**

Co-culture of three green microalgae, i.e. *Chlorella, Scenedesmus obliquus*, and *Ankinstrodomus sp* were isolated from Bojong Soang wastewater treatment plant in Bandung, Indonesia. This consortium cultured in vertical photobioreactor (total capacity 10L) as closed system were containing of 8 L Provasoli Haematocochus Medium (PHM) injected continuously during 12 days experiment, with various concentration pure CO_2 gas (0%, 2%, 5%, 7%) from the bottom of vertical column photobioreactor. Composition of PHM was (per litre): 1.0 g KNO_3, 0.2 g KH_2PO_4, 0.2 g MgSO_4.7H_2O, 0.1 g Fe stock consists of (per litre) 189 g EDTA and 24.4 g FeCl_3.6H_2O. All nutrients were dissolved in distilled water containing (per litre) 0.1 mL trace element made by (per 500 mL) 2.05 mg ZnCl_2, 30.5 mg H_3BO_3, 2.55 mg CaCl_2.6H_2O, 3.0 mg CuSO_4.5H_2O, 2.05 mg MnCl_2.4H_2O and 19 mg (NH_4)_6Mo_7O_24.4H_2O (Provasoli & Pintner, 1959). Environmental conditions adopted at 30°C temperature range with light intensity of 4000 lux obtained from fluorescent lamp, 16 hour light and 8 hour dark, 8 L/min of CO_2 flow rate.

**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 (m) of microalgae is calculated by the following formula:

\[ \mu = \frac{N_t - N_0}{t - t_0} \times \frac{1}{N_t} \hspace{1cm} \text{(Eq. 1)} \]

- \( m \) = specific growth rate (cell biomass/mL/unit time)
- \( N_0 \) = Initial cell culture density at time \( t_0 \) (cell/mL)
- \( N_t \) = Culture density at time \( t \) (cell/mL)
- \( t-t_0 \) = time interval (day)

Cell density (N) is obtained from calibration curve OD 665 vs X with a spectrophotometer.

**Measurement of CO_2 concentration**

Concentration of CO_2 in a series of photobioreactor system was measured twice 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.

**Determination of CO_2 removal and CO_2 removal efficiency**

Efficiency of CO_2 removal can be calculated by the following formula:

\[ \text{CO}_2 \text{ removal efficiency} = \frac{\text{Influent of CO}_2 - \text{Effluent of CO}_2}{\text{Influent of CO}_2} \times 100\% \hspace{1cm} \text{(Eq.2)} \]

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 (Ohtaguchi and Wijanarko, 2003 in Dianursanti, 2012).
Carbon Dioxide Fixation by Co-Culture Green Microalgae through Overall Volumetric Mass

$$CTR = \Delta y_{CO_2} \cdot \alpha_{CO_2}$$  \hspace{1cm} \text{(Eq.3)}$$

Where,

$$\alpha_{CO_2} = \text{a constant that contains a fixed number of temperature and pressure, airflow superficial velocity; } \Delta y_{CO_2} = \text{the concentration change of CO}_2 \text{ in and out of the reactor by the incoming CO}_2 \text{ concentration, multiplied by 100\%}$$

$$\alpha_{CO_2} = \frac{U_g \cdot A \cdot M_{CO_2} \cdot P}{V_{med} \cdot R \cdot T}$$  \hspace{1cm} \text{(Eq.4)}$$

Where, $U_g$ = superficial gas velocity, i.e. discharge gas fed per reactor cross-sectional area (m h$^{-1}$); $A$ = surface area of the photobioreactor facing or exposed to light (m$^2$); $M_{CO_2}$ = 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 ($^\circ$K).

Carbon fixation rate as specific CO$_2$ transfer rate ($q_{CO_2} \text{ gCO}_2/g \text{ cell/h}$) 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.

$$q_{CO_2} = \frac{\Delta y_{CO_2} \cdot \alpha_{CO_2}}{X}$$  \hspace{1cm} \text{(Eq.5)}$$

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

Measurement of volumetric CO$_2$ mass transfer coefficient ($K_a \text{ CO}_2$)

Carbon dioxide mass transfer coefficient ($K_a, \text{ CO}_2$) is a common parameter that is often used to assess the performance of the bioreactor in the microalgal cultivation process. The mass transfer of carbon dioxide from the gas phase to the liquid phase is expressed using the following equation:

$$Na = K_a \cdot (C_{*AL} - C_{AL})$$  \hspace{1cm} \text{(Eq.6)}$$

Where $Na$ is the rate of carbon dioxide transfer per unit of time (gmol m$^{-3}$ s$^{-1}$), $KL$ is the liquid phase mass transfer coefficient (m s$^{-1}$), $a$ is the gas-liquid interfacial area per unit volume of fluid (m$^2$ m$^{-3}$), CAL is the concentration of dissolved carbon dioxide in the broth, and $C_{*AL}$, also called the solubility of carbon dioxide, is the saturated concentration of carbon dioxide in the broth (gmol m$^{-3}$). The concentration difference ($C_{*AL} - \text{CAL}$) refers to the driving force triggering mass transfer of carbon dioxide from the gas phase to the liquid phase (Doran, 1995).

RESULTS AND DISCUSSION

Growth response vs CO$_2$ removal efficiency

The concentration of carbon dioxide in the culture medium should not be less than required in order to maximize growth but must not exceed the tolerance limits of inorganic carbon required. Figure 1 shows the co-culture can utilize CO$_2$ injected into the culture until it reaches concentrations of 7% and turn it into biomass. In a previous study (Rinanti et al., 2013) explained that 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. Sparging with microbubble allow thorough mixing, CO$_2$ mass transfer and also removes O$_2$ produced during photosynthesis. Bubbling carbon dioxide-rich gas through vertical photobioreactor not only provide CO$_2$ for microalgae, but also help deoxygenation suspension and mixing to increase the frequency of the cycle. Air is bubbled at the bottom—a strategy that provides good overall mixing, sufficient supply of CO$_2$, and efficient removal of O$_2$.

The reactivity of carbon dioxide in aqueous solutions establishes various equilibriums in its contact with water. The first equilibrium refers to the dissolution of the gas in the water, forming carbonic acid. The carbonic acid undergoes almost instantaneous dissociation into bicarbonate and carbonate ions with the total inorganic carbon concentration being given by the sum of the species.

![Fig. 1. CO$_2$ removal efficiency and dried weight biomass as a response of high concentration CO$_2$ supplied, 30°C, pH 6-7, flow rate of CO$_2$ of 8 L/min, 4000 lux of light intensity, 16 hour light/8 hour dark](Image)
CO$_2^-$, HCO$_3^-$ and CO$_3$(Carvalho et al., 2006).

Highest dry biomass of 2.70 g/L ($\mu = 0.38$) obtained from cultures supplied with 5% CO$_2$. The value was 2-fold higher than cultures supplied with 7% CO$_2$ ($\mu = 0.32$) but did not differ significantly with culture supplied with 2% CO$_2$ ($\mu = 0.11$). Our results are better than Ryu et al. (2009) study which produces a maximum dry weight of 2.02 g/L occurred in cultures supplied with 5% CO$_2$ while the minimum dry weight of 1.16 g/L occurred in cultures supplied with 0.5% CO$_2$. Results of our study, the dry weight of 1.40 g/L occurred in cultures supplied with 7% CO$_2$. Thus, it seems that an increase in the supply of CO2 will lead to an increase in dry weight biomass of microalgae, until a certain tolerance limits.

Tang (2011) using 5-20% CO$_2$ from flue gas as a carbon source for microalgae that cultured in a closed system. Although microalgae can be grown in culture were supplied with 5% to 20% CO$_2$ but the best growth potential occured in culture supplied with 10% CO$_2$. At high concentrations (> 20%), CO$_2$ will induce and cause the pH decreases, carbonate anhydrase activity was reduced, thus inhibiting the growth of microalgae. Previous research showed that the concentration of CO$_2$ over 5% can be harmful to cells and can be limited the growth of microalgae (De Morais and Costa, 2007, Yoo et al, 2011, Tang et al, 2011).

Fig. 1 also showed there were a linear relationship between the dry weight of the biomass to ability of microalgae for removing CO$_2$. Such as biomass dry weight, the data showed the highest removal efficiency of CO$_2$ (49.02%) shown by a culture that was supplied with 5% CO$_2$. The value shown 2-fold higher than efficiency in culture supplied by higher concentration (7%) of CO$_2$. Up to a certain limit of CO$_2$ concentration, the higher of biomass dry weight, the higher the efficient CO$_2$ removal. CO$_2$ absorption by microalgae cells supposedly stimulated by increasing the CO$_2$ content in the media, so absorption rate of CO$_2$ was low in the culture without the addition of CO$_2$ gas.

**Carbon dioxide transfer rate and Carbon dioxide fixation rate**

CO$_2$ transfer efficiency is one of the most important parameters to improve microalgae productivity and CO$_2$ biofixation in a photobioreactor culture system. Mass transfer of carbon dioxide in the photobioreactor will also be influenced by hydrodynamic aspects, one of which was superficial gas velocity in the photobioreactor. Superficial velocity ($U_g$, Eq. 4) also called volumetric flux, i.e. the gas volumetric flow rate divided by cross-sectional area photobioreactor. If the diameter of the photobioreactor was 15 cm, the surface area of the tube cross section was $1.767 \times 10^2$ m$^2$, the injected gas flow rate of 8 L / min so that the value of the superficial velocity of $7.5 \times 10^2$ m/s.

High concentrations of CO$_2$ supplied to the culture medium using a bubble as CO$_2$ transfer, proved to be more effective to promote growth, rather than doing the same thing at the ambient air. Fig. 2 showed the average of CTR fastest occured in cultures supplied with 5% CO$_2$. This value is not significantly different than the CTR occurred in culture that was supplied with 2% CO$_2$ but as well as cell dry weight and CO removal efficiency, the value of this 2-fold higher than the culture that was supplied with 7% CO$_2$. Value of carbon fixation rate did not differ significantly in any culture that were supplied with 2%, 5%, and 7% CO$_2$.

CTR values in all cultures increased from day 2 but gradually decreased from day 8 (Fig. 3A), whereas the value qCO$_2$ in all cultures begin to decline gradually beginning to the end of the study (Figure 3B). Culture supplied with 2% and 5% CO$_2$ showed CTR and q profile that did not differ value significantly, compared to CTR and qCO$_2$ profile in the culture supplied with 7% CO$_2$. Gas CO$_2$ flow rate of 8L/min was estimated to cause transfer and fixation of CO$_2$ to be less than the maximum. Low pH values and high aeration conditions due to the rapid flow rate causes a decrease in inorganic carbon available for CO$_2$ stripping. In addition, a
study shows the importance of pH control during inorganic carbon supplied to the growth of microalgae biofilm. Conversely, when the CO\(_2\) stripping lower production rate (for example, using low agitation and / or aerated conditions), the concentration of dissolved CO\(_2\) will increase in the value of saturation (saturated concentration) (Contreras, 2007).

Mass transfer of carbon dioxide capacity in the photobioreactor is determined by the liquid phase mass transfer coefficient and the specific area available for mass transfer (Carvalho et al., 2006). Mass transfer coefficient is very important because there is a close relationship between biological processes, physicochemical and CO\(_2\) concentration in the solution. This research resulted in mass transfer coefficient of 0.1491 per hour (0.4146.10^{-6}/sec) in cultures supplied with 5% CO\(_2\) (Table 1). This very small value was caused by the high aeration conditions due to the very rapid flow rate (8L/min), so diameter of bubbles being large and retention time in medium was going faster. Corresponding to increase in K\(_{La}\), initially specific growth rate increases but from the end of the transition zone, it starts decreasing. Shear stress may be possible reason for the fall in specific growth rate (Contreras,2007). Zhang et al. (2002) did the comparative analysis of K\(_{La}\) in different photobioreactor in different percent of CO\(_2\) and concluded that requirement of critical K\(_{La}\) increases with decrease in the concentration of CO\(_2\) in the inlet gas stream to meet the CO\(_2\) demand of microalgal cells.

Determination of mass transfer coefficient using the data saturation concentration of carbon dioxide (CO\(_2\) supplied to the culture) and have calculated the concentration in the water by using the Henry constant, where the Henry constant gas into a liquid was the 10^{-1.5} mol/L/atm (Michelcic, 1999). When there is mass transfer of carbon dioxide from the gas phase to the liquid phase (water), only dissolved CO\(_2\) was responsible for the mass transfer of carbon dioxide through the gas-liquid interface, though CO\(_2\) of all species were CO\(_3^{2-}\), HCO\(_3^{-}\) and CO\(_2\) (Kazim, 2012).

**CONCLUSION**

In considering the results of the studies described above, it would showed that the information about the overall mass transfer coefficient of carbon dioxide is essential for a better feeding control and accurate data of how much carbon dioxide has been consumed by microalgae in comparison to the

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**Table 1.** Carbon transfer rate (CTR; gr/L/h), Carbon fixation rate (q; per hour) and Mass Transfer Coeficient (K\(_{La}\); per hour) in elevated CO\(_2\) concentration

<table>
<thead>
<tr>
<th>Concentration of CO(_2) supplied</th>
<th>Carbon transfer rate (CTR; gr/L/h)</th>
<th>Carbon fixation rate (q; per hour)</th>
<th>Mass Transfer Coeficient (K(_{La}); per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>93.17</td>
<td>40.08</td>
<td>0.0438</td>
</tr>
<tr>
<td>5%</td>
<td>101.29</td>
<td>42.02</td>
<td>0.1491</td>
</tr>
<tr>
<td>7%</td>
<td>48.55</td>
<td>37.21</td>
<td>0.0251</td>
</tr>
</tbody>
</table>
supplied carbon dioxide. In terms of biomitigation, it means how efficient of carbon dioxide could be removed by microalgae. The highest $K_La$ value of 0.1491 per hour occurred in cultures supplied with 5% CO$_2$ in condition CO$_2$ gas flow rate of 8L/min. Increasing the value of $K_La$ will be pursued in next research to study the effect of gas CO$_2$ flow rate to CO$_2$ superficial gas velocity.

REFERENCES


