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UTILIZATION OF *Chlorella vulgaris* TO FIXATE A HIGH CONCENTRATION OF CARBON DIOXIDE IN A COMPOST-BASED MEDIUM

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ABSTRACT

Massive use of fuels by industry increase carbon dioxide (CO_2) emissions significantly. *Chlorella vulgaris* (*C. vulgaris*) is well known for its ability to fixate CO_2 and synthesize it to a lipid. As industry usually emits high concentrations of CO_2, it is necessary to investigate the behavior of microalgae in regard to CO_2 inflow. We studied cultivation of *C. vulgaris* in a photobioreactor (volume 18L) in a compost-basedmedium under illumination at 3000 lux for 90 hours. We show that initial cell density 0.137 $g \cdot dm^{-3}$ is able to fixate CO_2 up to 30.31 $g \cdot dm^{-3} \cdot day^{-1}$ (93.56%) under a CO_2 inflow of 23.80 $g \cdot hour^{-1}$ with biomass productivity 0.44 $g \cdot dm^{-3} \cdot day^{-1}$ and lipid yield 0.0795 $glipid \cdot gcell^{-1}$, and it also shows the potential to fixate carbon dioxide 28.43 $g \cdot dm^{-3} \cdot day^{-1}$ (31.51%) and produce high lipid amounts (0.0739 $g \cdot g^{-1}$) under a carbon dioxide inflow 48.17 $g \cdot hour^{-1}$. Cultivation with a higher initial cell density (0.325 $g \cdot dm^{-3}$) shows better resistance under carbon dioxide inflow 48.17 $g \cdot hour^{-1}$ with carbon fixation 37.95 $g \cdot dm^{-3} \cdot day^{-1}$ (58%), biomass production 0.82 $g \cdot dm^{-3} \cdot day^{-1}$, lipid yield 0.0834 $g \cdot g^{-1}$, and good potential under carbon dioxide inflow 65.96 $g \cdot hour^{-1}$. This research shows the potential of *C. vulgaris* in reducing high concentrations of CO_2, which is beneficial for biomass and/or lipid production. These are in turn useful for biodiesel and food supplements. Further study is necessary for adapting this potential on a commercial scale.

Keywords: Carbon dioxide; Chlorella vulgaris; Fixation; Initial cell density

1. INTRODUCTION

Global warming has reached alarming levels (Kumar et al., 2011). The CO_2 emissions have reached a level of 32 gigaton in 2013, and it keeps increasing by 3.6% each year (IEA, 2014). Carbon capture technology is still far from being viable, therefore an applicative and cheap fixation method is urgently needed (Rossi et al., 2015). In this regard, microalgae have been well known for their ability to fixate the industrial emissions gases (15% v/v CO_2; Kaiwan-arporn et al., 2012), due to their cellular photosynthesis processes that convert CO_2 to beneficial glucose and lipid. Furthermore, biomass and lipid produced from the emissions gases could be further synthesized commercially into food supplements, colorants, and/or biodiesel. Microalgae possess a higher level of photosynthesis efficiency (6-8% on average), in comparison with terrestrial plants (reaching only 1.2-2.2%) (Aresta et al., 2005), therefore they are an important choice for the bioprocessing of the CO_2 emissions, hence reducing global warming effects.

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Upon using bioprocessing of microalgae to fixate industrial emission gases, several parameters determining the fixation rate are the subject of investigation and of interest. Numerous researches have been focusing on reactor designs, medium selections, and microalgae strains. One of the most important aspects is concentration of CO_2 inflow; where a high concentration of CO_2 might slow down or even inhibit the growth of microalgae, whereas a low concentration reduces the growth. The effect of CO_2 concentration becomes an important factor to be evaluated, especially since such an evaluation would resolve allowable concentrations of industrial emissions gases, which are usually high. These gases thenflow into a reactor for optimum fixation.

In this study, we investigated the fixation of CO_2 in bioprocesses employing microalgae *Chlorella vulgaris* (*C. vulgaris*). We have selected *C. vulgaris*, among other strains, due to its high tolerance inCO_2 (15-100% v/v; Li et al., 2013). Thus, this study would provide further analysis on the fixation of CO_2 and its effect on the growth of microalgae. To our knowledge, this finding is an advanced report on the effect of CO_2 concentration on the growth rate of *C. vulgaris* in an organic fertilizer-based medium.

2. EXPERIMENTAL SETUP

C. vulgaris used in the study was provided by Chemical Engineering Laboratories, Universitas Indonesia. The algae were cultured in 18 L flat plate reactor (0.4m×0.5m×0.1 m) (Dianursanti, 2012) with bubble purging through a cylindrical modified pipe at aconstant temperature (29ºC) and an ambient pressure (1 atm), containing a diluted commercial organic fertilizer medium. Aeration flow rate is controlled at 1.2 $L \cdot min^{-1}$ for various concentrations of CO_2 inflow. The reactor was incubated under continuous illumination 3.000 lx from a single front face (0.4m×0.5m) for 90 hours. The illumination source used was six parallel Philip Halogen (20W/12V/50Hz) lamps.

Six different reactors containing *C. vulgaris* were prepared. Four of them were prepared with an initial cell density of 0.137 $g \cdot dm^{-3}$, but different concentrations of CO_2 inflow. Hereafter the reactors are denoted as Reactor [A]: 0.03 $g \cdot hour^{-1}$, Reactor [B]: 23.80 $g \cdot hour^{-1}$, Reactor [C]: 48.17 $g \cdot hour^{-1}$, and Reactor [D]: 65.96 $g \cdot hour^{-1}$, respectively. The other two reactors were prepared with an initial cell density of 0.325 $g \cdot dm^{-3}$ and different concentrations of CO_2 inflow, denoted as Reactor [C']: 48.17 $g \cdot hour^{-1}$, and Reactor [D']: 65.96 $g \cdot hour^{-1}$. The prepared reactors were then irradiated under constant illumination to investigate the amount of biomass and lipid produced. Biomass density was evaluated using spectrophotometer UV-Vis (LaboMed Inc.) at 600 nm wavelength for *C. vulgaris*. Cells were obtained through vortex centrifugation beforehand and then disrupted using sonicator and microwave radiation. Lipids were extracted using the Bligh Dryer method and then measured by mass difference.

3. RESULTS AND DISCUSSION

Figure 1 shows the average fixation fraction of the prepared reactors. A fraction of fixation of CO_2 is obtained by calculating the difference between initial and final CO_2 concentrations left in the reactor relative to the initial CO_2 concentration. Thus, the fraction of fixation is in between 0 to 1, related to zero and complete fixation, respectively towards CO_2 inflow.

The highest fraction (93.57%) was reached for Reactor B in which the CO_2 inflow is 23.80 $g \cdot hour^{-1}$. It is higher than that of Reactor A, having a CO_2 inflow of 0.03 $g \cdot hour^{-1}$. This indicates that increasing supply of CO_2 in the medium leads to higher fixation ability. It may be related with the growth of the microalgae, where CO_2 acts as carbon source for photosynthesis of *C. vulgaris.* It is in accordance with theory of growth, where availability of substrate affects the growth positively (Sheets et al., 2014). On the other hand, a higher concentration of CO_2

inflow, as shown in Reactors C and D, shows an opposing effect towards fixation fraction, suggesting that there is a limitation in the fixation ability of *C. vulgaris* to convert CO_2 to glucose and/or the solubility of CO_2 into a liquid phase medium.

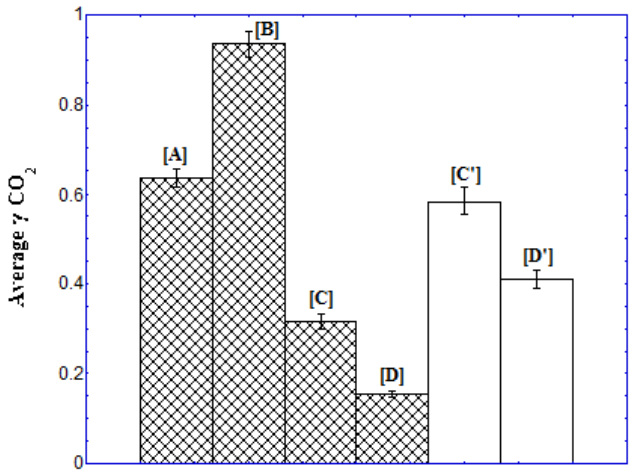

Figure 1 Average fixation fraction of each variation

Higher cell density shows a better fixation fraction at a similar CO_2 inflow concentration. This means that the initial cell density plays an important role in the fixating of CO_2. It is suggested that in a higher cell density, there are more cells available to process CO_2 into useful compounds through photosynthesis, as long as light irradiation is accessible (Dianursanti et al., 2014).

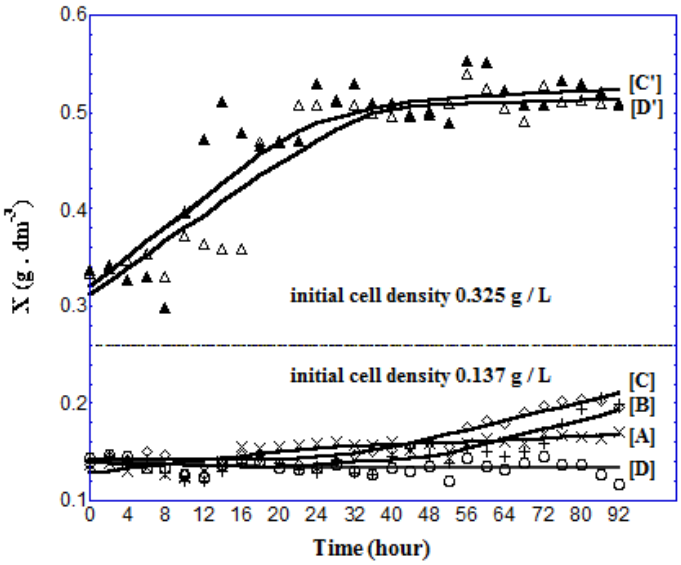

Figure 2 Time-dependent cell density from each variation

Figure 2 shows time-dependent cell density observed within 90 hours of cultivation. It is seen that in contrast to the results in fixation fraction, in low initial cell density (0.137g/L) Reactor C shows the highest cell density increase, followed in sequence by Reactors B and A. This result confirms the theory of cell growth. However, Variation D (carbon concentration inflow 65.96 $g \cdot hour^{-1}$) shows relatively no growth caused by the high rate of CO_2 input. This may also be attributed to the kinetic model prediction offered by Wijanarko et al. (2004) for *C. vulgaris*,

where this microalgae follows the kinetic model of Haldane which includes substrate inhibition, shown by the following growth rate in Equation 1.

$$\mu = \mu_{maks} \cdot \frac{[HCO_3^-]}{K_S + [HCO_3^-] + \frac{[HCO_3^-]^2}{K_I}} \quad (1)$$

μ represent a growth rate, μ_{max} for maximum growth rate, $[HCO_3^-]$ for bicarbonate ion concentration. It also shows Ks represents a substrate constant and K_I is for the inhibition constant. The inhibition occurs when the concentration reaches a certain level. This current study suggests the inhibition level is in-between 48.17 g/h and 65.96 g/h for the initial cell density of 0.137 $g \cdot dm^{-3}$ under similar operating conditions.

As mentioned above, fixation fraction and cell growth show different tendencies. This phenomenon reflects the limitation of CO_2 which could be fixated by microalgae. The overall average fraction of Reactor C is indeed low, but the numerical value of fixated CO_2 (28.43 $g \cdot dm^{-3} \cdot day^{-1}$) is not far from Reactor B (30.31 $g \cdot dm^{-3} \cdot day^{-1}$). The small fraction of fixated CO_2 is likely due to a high initial CO_2 concentration.

Variation A shows growth of microalgae with outdoor air. In comparison, Reactor A shows a shorter lag phase, which is a period for microalgae adaptation to environment, rather than in Reactors B and C. Thus it is reasonable that Reactor A shows no significant growth. Thelag phase, on the other hand, also indicates an adaptation phase period when the microalgae uses available substrate to grow. This finding suggests that the presence of CO_2 concentration can lengthen the lag phase (Richmond, 2004) and decrease the acidity of the medium, leading to a condition in which the microalgae need to adjust themselves.

In a higher initial cell density culture, the log phase is significantly short, as shown in Figure 2. This suggests that higher initial cell density shortens the adaptation phase, due to high number of cells. Thus, denser population increases both physical contact between cell membrane and bicarbonate ion, as nutrition demand. As a result, the bicarbonate ion surrounding individual cell membranes isvalued at a smaller rate, thus the substrate inhibition effect is reduced.

Cultivation under initial cell density 0.325 $g \cdot dm^{-3}$ (Reactors C' and D') also shows the high slope of growth curve, indicating a high yield of cells. It is suggested that once the adaptation phase is passed, microalgae chlorophyta consume bicarbonate ions and uses carbon atoms to synthesize metabolic compounds, such as glucose, lipid, and cell organelles. Dianursanti & Santoso (2015) suggested that depending on the availability of nitrogenous compounds, carbon is synthesized into chloroplast. This is supported by a compost based medium, which is proven to contain nitrogenous compounds.

CO_2 fixation relies also on its dissolution rate. Microalgae fixate carbon sources in the form of bicarbonate ions (Dianursanti, 2012). Thus, it is important to evaluate the profile of the bicarbonate ion. The concentration of bicarbonate ions are calculated by

$$[HCO_3^-] = \left(\frac{KCO_2}{HCO_2}\right)\left(\frac{yCO_2 \cdot P_T}{10^{-pH}}\right)\left(\frac{EXP\left[A_k\left(1-\frac{T_0}{T}\right)+B_k \ln\left(\frac{T_0}{T}\right)+C_k\left(\frac{T}{T_0-1}\right)\right]}{EXP\left[A_h\left(1-\frac{T_0}{T}\right)+B_h \ln\left(\frac{T_0}{T}\right)+C_h\left(\frac{T}{T_0}-1\right)\right]}\right) \quad (2)$$

$[HCO_3^-]$ stand for the bicarbonate ion concentration, K CO_2 stands for steady state constant of CO_2 (4.38×10^{-7}), H CO_2 for Henry Constant (2900 KPa mol^{-1}), y CO_2 for carbon dioxide concentration (gram dm^{-3}), P_T for operating pressure (atm), T for operating temperature (K) and pH for acidity. In the other hand A_k, B_k, C_k, A_h, B_h and C_h stand for the gas activity constant

with values of 40.557, -36.782, 0.000, 22.771, -11.452, and 3.117, respectively in sequence. The equation shows that the concentration of the soluble bicarbonate depends on the pH level, Dianursanti (2012). Thus, pH is measured to find the solubility of the bicarbonate ions. The time evolution of both the pH and bicarbonate ions is shown in Figures 3 and 4, respectively.

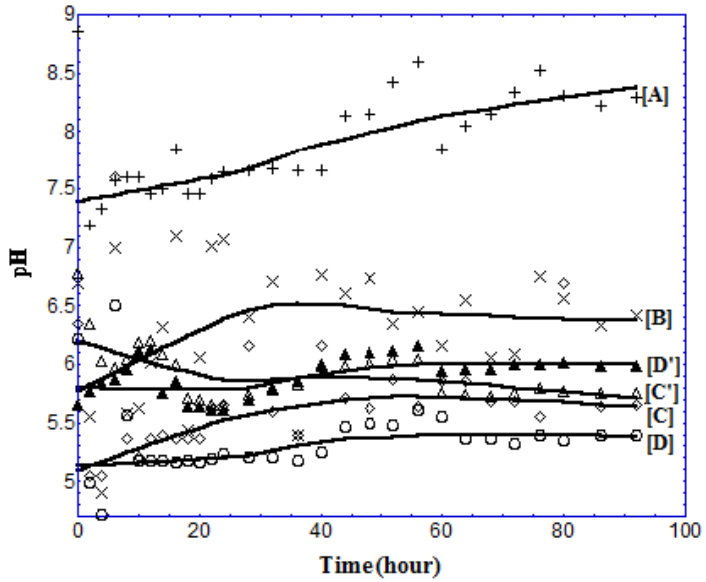

Figure 3 pH profile of each variation

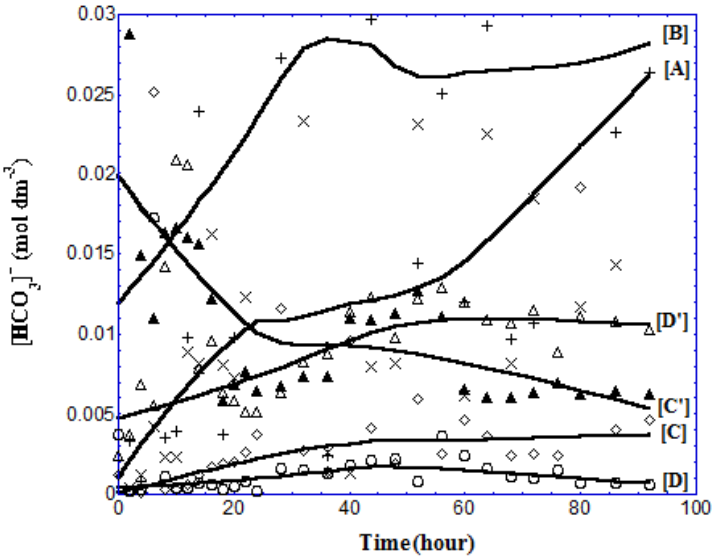

Figure 4 Bicarbonate ion concentration of each variation

As shown in Figure 3, the addition of CO_2 decreases the pH level and thedissolution of CO_2 into bicarbonate ions, which increase the concentration of the hydrogen ion, decreasing the pH. In Reactor A, with practically no addition of CO_2 (utilizing the atmospheric concentration of CO_2), the pH increases steadily. This can be attributed to the metabolism of the microalgae which tends to increase the pH (Dianursanti & Santoso 2015). This strongly suggests that the increase of pH occurs due tothe decreasing concentration of carbon and nitrogen sources, two compounds contributing in acidity.

Further, Figure 3 indicates that higher CO_2 flow in leads to a larger decrease in pH. Thus, for Reactors C and D, which contain a relatively higher concentration of CO_2 than that in Reactor B, their pH levels are lower. However, there is a limit on the dissolution of CO_2; as shown by Reactors C and D, during which the time evolutions of the pH levels are similar to each other, where the concentration of the bicarbonate ions in the two reactors is limited by fluid mechanics and cell activity. High cell activity allows a higher CO_2 concentration gradient between bulk and cell membranes. This concentration gradient becomes an influential driving force, as predicted by Wijanarko et al. (2004).

Cultivation with higher initial cell density shows relatively stable pH, indicating higher cell activity in consuming bicarbonate ions in attempting to balance out CO_2 dissolution from gas bubble form to medium bulk in comparison with their counterparts in the lower cell density. Reactor D possesses lower cell activity, creating a lower gradient, thus inducing saturation faster than that of Reactor D', which shows a steady increase of HCO_3^- concentration up until 40 hours. This finding also indicates that a higher initial cell density shows a higher consumption of bicarbonate ions per cell.

Figure 4 confirms the above explanation. We could observe an increasingly dissolved concentration of the bicarbonate ions as shown by both Reactors A and B. This is likely due to high photosynthetic activity, thus increasing demand for the carbon source. In comparison to Reactors C and D, the bicarbonate ion concentration is relatively low due to low photosynthetic activity. On the other hand, Reactors C' and D' show better photosynthetic activity, as confirmed by better growth in both instances, (See Figure 2) as well as higher bicarbonate ion concentration (See Figure 4). This shows that higher initial cell density can withstand the higher carbon dioxide concentration.

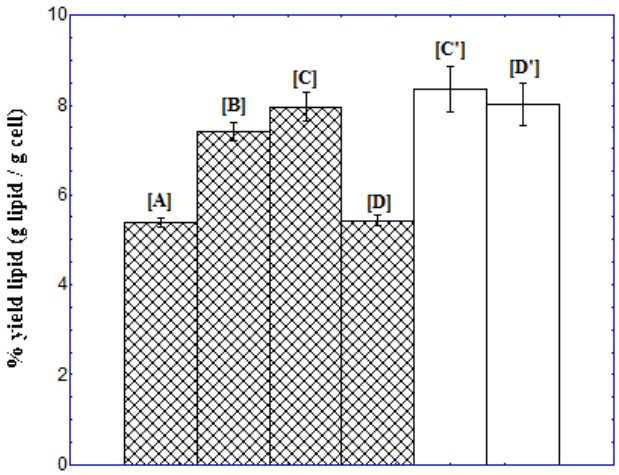

Figure 5 lipid percentage yield of each variation

Figure 5 shows the lipid yield of each reactors. An increase in CO_2 concentration is followed by an increase in lipid yield. Reactor D, shows very low lipid yield as low as that in Reactor A. It is suggested that under a very high CO_2 concentration, the microalgae metabolism and the lipid synthesis pathway are inhibited (Dianursanti & Santoso, 2015). The highest cellular lipid is found in Reactor C, followed by Reactor B. This is in accordance with the carbon fixation model offered by Moheimani (2005). He suggested in his dissertation that the bicarbonate ion and CO_2 could be consumed by a hydrogen ion to balance the reaction. The hydroxide ion is also useful in facilitating dark cycle in the Rubisco and polysaccharide synthesis reaction in Golgi vesicle as a packaging organelle. In the specific model, it is suggested that Reactors B and C experience a large amount of bicarbonate supply, which induces the lipid mechanism to

be dominant. Thus, Reactor C which has a larger amount of bicarbonate supply, results in a larger synthesized cellular lipid. The hydroxide ion in this model becomes excessive, allowing the lipid to be continuously synthesized in the cell.

Reactors C' and D' show high cellular lipid content for several reasons. One of the reasons is higher bicarbonate consumption, which may induce a faster metabolism pathway in synthesizing cellular lipid. Another reason is that higher initial cell density increases the self-shading mechanism, triggering microalgae to synthesize lipid as a food reserve in comparison to glucose. Also, the right amount of carbon dioxide will run chlorophyta to consume bicarbonate ions and use the carbon atom to synthesize metabolic compounds such as glucose, lipid, and cell organelles.

4. CONCLUSION

We concluded that CO_2 concentration has a relatively positive effect on increasing the lipid yield and efficiency of CO_2 fixation up to a certain limit. Further study on a denser cell population could be utilized to reach a higher fixation fraction and lipid yield.

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