MICROALGAE BIOMASS PRODUCTIVITY BY CO_2 INJECTION IN CORRELATION WITH pH VARIATION IN PHOTOBIOREACTOR

PRODUKTIVITAS BIOMASSA MIKROALGAE DENGAN INJEKSI CO_2 YANG BERKORELASI DENGAN VARIASI pH DALAM FOTOBIOREAKTOR

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ABSTRAK

Mikroalgae dapat memberikan solusi bagi reduksi CO_2 dan sebagai sumber energy alternatif yang berkelanjutan. Reduksi CO_2 oleh mikroalgae dilakukan dengan adanya fiksasi CO_2 selama proses fotosintesis terjadi. Tujuan dari penelitian adalah untuk mendapatkan kondisi pH pengaruh dari injeksi CO_2 dalam kultivasi microalgae yang menghasilkan produktivitas biomassa lebih tinggi. Mikroalgae yang digunakan adalah *Scenedesmus*.sp. *Scenedesmus*.sp dapat tumbuh dengan baik dalam media Walne saat dilakukan eksperimen skala laboratorium dibanding dengan media yang lain. Kontrol pH yang dilakukan dengan memberikan injeksi CO_2 menunjukkan perolehan berat kering tertinggi yaitu pada nilai pH dengan kisaran 7,0-7,5. Sebagai salah satu faktor pengontrol yang menentukan kemampuan biologis mikroalgae dalam menggunakan nutrisi yang diserap, nilai pH pada kisaran 8,0 – 8,5 dan 9,0 – 9,5 dapat menurunkan proses fotosintetis di dalam mikroalgae *Scenedesmus*.sp. Sehingga diperoleh hasil fiksasi CO_2 yang tertinggi juga terjadi pada pH kisaran 7,0 – 7,5. Penelitian ini memberikan penjelasan bahwa pada kisaran pH 7,0 – 7,5 terkandung lebih banyak CO_2 daripada kisaran pH 8,0 – 8,5 and 9,0 – 9,5. Temperatur kultur microalgae tidak menunjukkan fluktuasi yang menyimpang jauh pada pengamatan yang dilakukan di pagi maupun siang hari.

Kata kunci: mikroalgae, *Scenedesmus*.sp, injeksi CO_2

ABSTRACT

Microalgae can bring solution for reducing CO_2 and as alternative sustainable energy source. CO_2 reduction by microalgae was done by fixation of CO_2 during photosynthesis while they grow. The objective of the study is to get pH condition from CO_2 injection into cultivated microalga that gives better productivity of biomass. Microalga that used was Scenedesmus.sp. Scenedesmus.sp did grown better in Walne media on lab scale experiments. CO_2 injection as pH control that gave the highest dry weight obtained on pH range value 7.0-7.5. As one of the controlling factor that determines the biological ability of microalgae in utilizing nutrient, pH value shows that at range of pH 8.0 – 8.5 and 9.0 – 9.5 can decrease photosynthetic process on microalgae Scenedesmus.sp Thus the highest fixated CO_2 also on pH value 7.0 – 7.5. This research give a brief explanation that in the range of pH 7.0 – 7.5 contain more CO_2 than range pH 8.0 – 8.5 and 9.0 – 9.5. Culture temperature did not show any high fluctuation at morning and in the mid-day of treatment.

Keywords: microalgae, *Scenedesmus*.sp, CO_2 injection,

I. INTRODUCTION

There are many researchers pay attention on reducing CO_2 emissions and developing renewable and sustainable energy sources as alternatives to fossil fuel. Microalgae have a potential solution for both issues. Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bio actives

(Chisti, 2007). The idea of using microalgae as a source of fuel is not new, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005). Microalgae based CO_2 fixation is considered one of the most effective and environmental-friendly approaches to reduce atmospheric CO_2 (Ho et al. 2011). Microalgae have a 10-50 times higher growth rate than terrestrial plants, thus giving an extremely high CO_2 fixation rate (Wang et al. 2008).

Microalgae need nutrients that consist of macronutrient and micronutrient. Examples of macro-nutrients for the growth of microalgae are inorganic compounds such as N, K, Mg, S, P and Cl. Micro nutrients are Fe, Cu, Zn, Mn, B, and Mo (Ilavarasi et al, 2011). Cultivated microalgae concentration is generally higher than that in the wild. Nitrate, phosphate and silicate nutrients added in the cultivation of algae to meet the nutrient medium cultivation (Støttrup & McEvoy L, 2008).

In microalgae growth pH is an important parameter. According to FAO, the pH range value for most of the microalgae is 7 – 9, and their optimum pH range value depends on microalgae species. At Wu et al. (2008) indicate that in media that initially with lower pH value, then the pH will increase due to the process of photosynthesis. Whereas if the initial pH close to 8.5 the pH value will stabilized in the next 2 – 3 weeks. At high pH values, the population of algae (diatoms) will be able to adjust to the high pH state so there is no drastic decrease in the pH value. pH value is also a controlling factor that determines the biological ability of microalgae in utilizing nutrient. The photosynthetic activity of microalgae, for example will reduce when pH value is too high.

In a closed system, a steady decrease nutrient is accompanied by a change in pH and alkalinity. Changes in pH and alkalinity are the results of net fluxes of H^+ across the plasma membrane as well as influx different dissociation states of weak electrolytes such as CO_2/HCO_3^- and NH_4^+/NH_3 (Granum & Myklestad, 2002). During algal growth, fluxes of inorganic and organic species occur between compartments inside the cells and the surrounding medium (Granum and Myklestad, 2002).The chemical equilibrium between inorganic carbon and protons constitute a natural pH buffering system in water as described by the following equations (Wu et al, 2008).

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$$

The ability of algae for CO_2 fixation can also be an interesting method for removing gases from power plants, and thus can be used to reduce greenhouse gases with a higher production micro algal biomass and consequently higher biodiesel yield. Microalgal biomass contains approximately 50% carbon by dry weight (Sánchez et al. 2003). All of this carbon is typically derived from carbon dioxide. Producing 100 tons of microalgae biomass fixes roughly 183 tons of carbon dioxide. Microalgae biomass production systems can be easily adapted to various levels of operational and technological skills as biodiesel production with high oxidation stability. The objective of the study is to get pH condition from CO_2 injection into cultivated microalga that gives better productivity of biomass.

II. METHODOLOGY

A. Microalgae Media Selection

The microalgae used in this study were *Scenedesmus* sp wild type. Microalgae were cultivated in 4 different nutrient medium, ie Yashima, MN, Walne and Allen Miguel.

- Yashima consists of: Urea 0.02 g, ZA 0.2 g, TSP 0.02 g in 2 liters sterilized distillated water.
- MN consists of: Na_2EDTA 0,016 g/l, $MgSO_4.7H_2O$ 0,25 g/l, KNO_3 1 g/l, $FeSO_4.7H_2O$ 0,002 g/l. Add trace element 1ml/liter that consists of H_3BO_3 2.86 g/l, Na_2MoO_4 0.021g/l, $CuSO_4$ 1.8 g/l, $MnSO_4$ $4H_2O$ / $MnSO_4$ H_2O 1.3 g/l / 0.985 g/l, $ZnSO_4 \cdot 7H_2O$ 0.22 g/l.
- Walne as ready to use media from Varicon Aqua Solutions as Cell Hi-WP
- Allen Miguel consists of: Solution A 2 ml/l: KNO_3 20 g/100ml.

  Solution B 1 ml/l: $Na_2HPO_4 12H_2O$ 4 g, $CaCl_2.6H_2O$ 2 g, $FeCl_3$ 2 g, HCl 2 ml, sterilized distillated water 80 ml.

All the microalgae tested were grown in erlenmeyer 500 ml volume with aeration bubbling under lamp lighting conditions, at the optimal temperature for microalga *Scenedesmus* sp, The growth parameter such as optical density (OD) (680 nm) was measure

using spectrophotometer Genesys-10uv series from Thermo scientific.

B. pH Control

Microalgae were grown in photo bioreactor from Bio-Fence™ as shown in Fig.1 which has 800 liters volume capacity. Microalgae will be pumped from the tank through transparent tubular tube and back again to the tank. Microalgae cultivation would injected by CO_2 that triggered by pH change. pH control was set between lower limit and upper limit i.e.: 7.0 – 7.5; 8.0 – 8.5; and 9.0 – 9.5. When pH measurements exceed the upper limit because of the microalgae metabolism, it will trigger valve to open CO_2 injection. CO_2 injection into microalgae cultivation will decrease pH value. Decreasing pH value below lower limit will automatically stop CO_2 injection.

The dry weight of *Scenedemus*.sp microalgae was obtained by calculating the optical density by the following equation:

Dry weight (gram)=(OD-0,005)/1934)×1000

C. CO_2 Fixation

Calculation of CO_2 fixation was carried out through the photosynthesis reaction equation:

$$6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_6$$

The amount of CO_2 consumed by microalgae will be used as the base of calculation

- Carbohydrate concentration was calculated by following equation

  Carbohydrate = Microalgae dry weight x 35.07%

  35.07% is from sun dried treatment

  Referents: Procsimate Analysis using AOAC Methods (1999), AOAC, 1999, Official Methods of Analysis, 13rd Ed, Association of Official Analytical Chemist, Washington DC

- Glucose ($C_6H_{12}O_6$) = Carbohydrate × $^{9}/_{10}$

  Referents: (Abdulgani Nurlita, 2010)

- Using Glucose value, CO_2 can be calculated by equation below:

$$\text{Mol } C_6H_{12}O_6 \text{ (A)} = \frac{gram\ C_6H_{12}O_6}{Molecules\ weight\ C_6H_{12}O_6}$$

$$\text{Mol } CO_2 \text{ (B)} = {}^{6}/_{1} \times A$$

Figure 1
Closed system photo bioreactor Bio-Fence™

CO_2 Consumption = $B \times CO_2$ *molecule weight* $\times 99.9\%$

(note: 99.9% is CO_2 concentration that used)

Temperature measure is temperature probe in photo bioreactor which is shown on a control panel of the bioreactor.

III. RESULTS AND DISCUSSION

A. Microalgae Media Selection

The objective of the medium nutrient selection for microalgae is to get the best nutrient for microalgae growth that will be used as medium on photo bioreactor. Culture medium is an important factor on microalgae utilization. The selected media culture is a media culture containing macro nutrition and micro nutrition that is needed by microalgae which grows. A complete composition and proper concentration of nutrition determine biomass production and nutritional content of microalga (Prihantini et al. 2007). Microalgae growth always shows sigmoid curve. The sigmoid curve is a 'growth average' representing all organisms, where young organisms experience adaptation phase and then rapid accelerating growth to cope with their environment, followed by a continuous steady growth. Towards maturity, the growth rates of organisms' slow down until no growth occurs. At the beginning, microalgae need more adaptation used Walne media, but after that its growth faster than the others. Fig. 2. Shows that each of nutrient media variation have similar trend, microalga still on exponential phase at the end of experiments, except for Allen Miguel media that already shows steady growth and start to decrease.

From the experiments shows microalgae growth in 4 different nutrient medium. The highest growth is microalgae that use walne nutrient medium, followed by Yashima at second, MN and last Allen Miguel nutrient medium. The Walne media brought the best growth for *Scenedesmus*.sp because it contained nutrient that fit for *Scenedemus*.sp growth than others. *Scenedesmus*.sp can absorbed Walne culture media better than others nutrient media. Good nutrients absorption of *Scenedesmus*.sp shown by optical density as Fig. 2, the density of microalgae measured. Walne media in the end of observation gave OD value: 0.818, yashima media: 0.671, MN media: 0.584, and Allen Miguel media: 0.331.

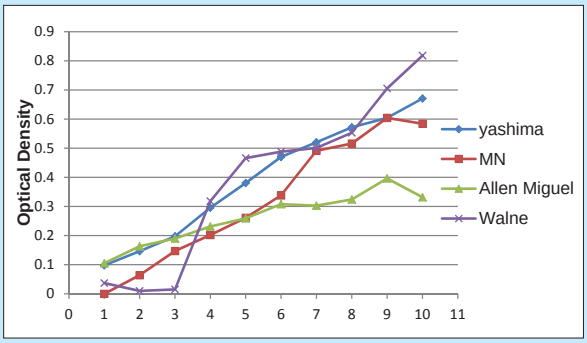

Figure 2
Optical density of *Scenedesmus* sp in different nutrient medium

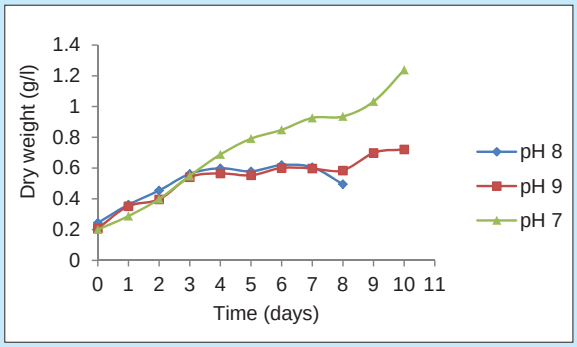

Figure 3
Microalgae Scenedesmus sp. growth based on dry wight at each pH variation

B. pH Control

In photo bioreactor, microalgae was use walne growing media as the best nutrient. This nutrient media is more soluble and easy to use in bigger scale than other medias.

During experiments for 10 days observations microalgae *Scenedesmus*.sp dry weight that obtained on pH range 7.0 – 7.5 is the highest than pH range 8.0 – 8.5 and 9.0 – 9.5. The dry biomass that obtained from harvesting microalgae at range of pH 7.0 – 7.5 were 1.24 g/l, pH range 8.0 – 8.5 0.495 g/l dry biomass and pH range 9.0 – 9.5 0.72 g/l dry biomass. The growth curve of microalgae *Scenedesmus*.sp during experiments still shows an exponentials phase at range of pH 7.0 – 7.5 and 9.0 – 9.5, but growth curve at range pH 8.0 – 8.5 already shows decreased of microalgae *Scenedesmus*.sp. CO_2 injection could decreased pH value down to 5 but this pH change only have slight influenced on microalgae growth (Maeda et al. 1995).

Mikroalgae *Scenedesmus*.sp growths on range of pH value 8.0 – 8.5 increased till day 3rd and then continue in steady phase and begin to decrease at day 7th. Microalgae at range pH 9.0-9.5 have the same pattern of growth factor that increase till day 3rd and continue in steady phase but still have increased till day 10th. Microalgae *Scenedusmus*.sp that growth in pH range 7.0 – 7.5 seems have stabilized growing curve than range pH 8.0 – 8.5 and 9.0 – 9.5 (see Fig.3). This might at range of pH 7.0 – 7.5 is a suitable pH value to grow for microalgae *Scenedesmus*.sp. As one of the controlling factor that determines the biological ability of microalgae in utilizing nutrient, pH value shows that at range pH 8.0 – 8.5 and 9.0-9.5 can decrease photosynthetic process on microalgae *Scenedesmus*.sp.

C. CO_2 Fixation

Microalgae from Chlorophyceae Classes have green pigment (chlorophyll) that can perform photosynthesis. In the photosynthesis, CO_2 is required as a raw material for the formation of the metabolites and biomass. Microalgae have a relatively fast growth rate, so it needs quite high CO_2 to fit their needs. Thus, microalgae suitable for use as a carbon sink to help decrease the levels of CO_2 in the air. CO_2 is the main ingredient for photosynthesis, in which the speed of photosynthesis and CO_2 consumption increasing with the increase of available CO_2 concentration. One of the important factors that affect this phenomenon is the pH of microalgae medium. (Wu et al, 2008).

CO_2 fixation by *Scenedesmus*.sp microalgae shows best result on pH 7.0-7.5 as shown at Fig.

4. CO_2 fixation in pH range of 7.0 – 7.5 is 0.471 gram, in 8.0 – 8.5 0.114 gram and 0.233 gram in pH range 9.0 – 9.5. According dry weight biomass, CO_2 fixation also gives the same result, because calculated based on dry weight biomass. This means the bigger dry weight biomass is from better CO_2 fixation of microalgae. CO_2 was used as raw materials to produce carbohydrates that are needed by microalgae to grow and maintain their life. *Scenedesmus*.sp could grow under 80% CO_2 conditions but the maximum cell mass was observed in 10-20% CO_2 concentrations ((Gordon & Seckbach 2012).

CO_2 fixation is dependent on source of light, CO_2 and water. This research gives a brief explanation that in the range of pH 7.0 – 7.5 contains more CO_2 than the range of pH 8.0 – 8.5 and 9.0 – 9.5. It is said chemical equilibrium between inorganic carbon and protons constitute a natural pH buffering system in water. Carbon dioxide dissolves slightly in water to form a weak acid called carbonic acid, H_2CO_3, according to the following reaction:

$$CO_2 + H_2O <=> H_2CO_3$$

After that, carbonic acid reacts slightly and reversibly in water to form a hydronium cation, H_3O^+, and the bicarbonate ion, HCO_3^-, according to the following reaction:

$$H_2CO_3 + H_2O <=> HCO_3^- + H_3O^+$$

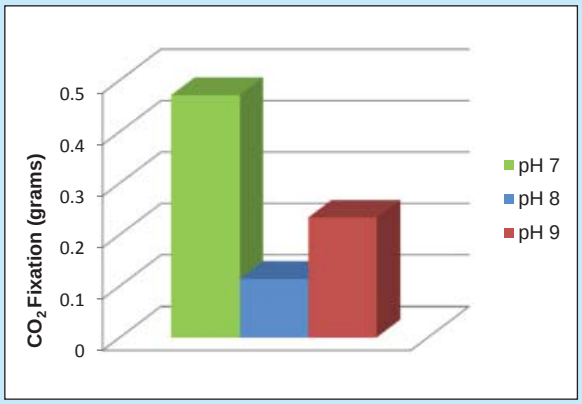

Figure 4
CO_2 fixation by *Scenedesmus*. sp during experiments

D. Temperature Fluctuation

Culture temperature range in the morning is around 27º – 37º C. During observation of the microalgae *Scenedemus*.sp culture on pH range 7.0 – 7.5 the temperature has slight fluctuation, compared to temperature microalgae culture that set on pH range 8.0 – 8.5 and 9.0 – 9.5 (see Fig. 5). While culture temperature range in the mid-day about 32º – 40º C. Microalgae *Scenedesmus*.sp usually can grow optimally at 28º – 30º C. *Scenedesmus*.sp that is used in this research growth in culture temperature range 27º – 40º C, that is higher than their optimal temperature. Culture temperature can also influence microalgae growth becomes less than when they grow in their optimal temperature.

IV. CONCLUSION

The best media nutrient is Walne media and followed by Yashima media, MN and last Allen Miguel media. Microalgae Scenedesmus.sp has better growth in pH range 7.0 – 7.5 that controlled by CO_2 injection. It is evidenced by dry weight that was

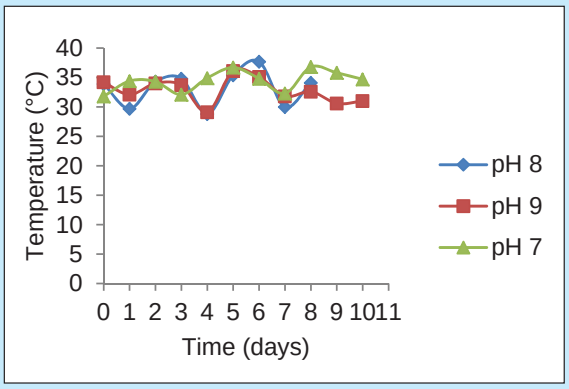

Figure 5
Culture Temperature fluctuation in the morning

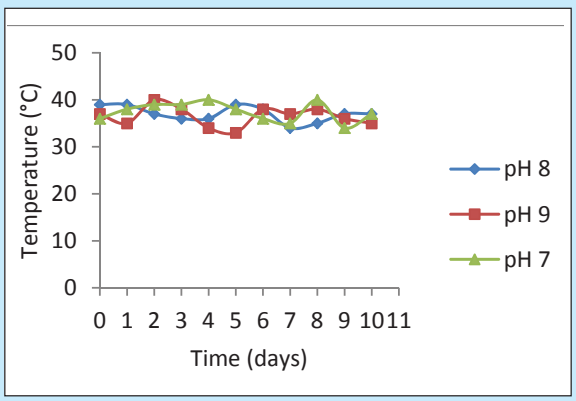

Figure 6
Culture temperature fluctuation in the mid-day

obtained is the highest. CO_2 fixation at pH range 7.0 – 7.5 is higher than 8.0 – 8.5 and 9.0 – 9.5 at value 0.471 gram CO_2 during observations. Culture temperature had only slight fluctuation but had higher on range average temperature than its optimal temperature.

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