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Enhancement of Carbon Dioxide Fixation by Alteration of Illumination during *Chlorella vulgaris*-Buitenzorg's Growth

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Abstract Alteration of illumination with optimum carbon dioxide fixation-based curve in this research successfully enhanced the CO_2-fixation (q_{CO2}) capability of *Chlorella vulgaris* Buitenzorg cultivated in a bubble column photo bioreactor. The level of CO_2 fixation was up to 1.91 times that observed from cultivation with intensification of illumination on an optimum growth-based curve. During 144 h of cultivation, alteration of light intensity on an optimum CO_2-fixation-based curve produced a q_{CO2} of 12.8 h^{-1}. Meanwhile, alteration of light intensity with a growth-based curve only produced a q_{CO2} of 6.68 h^{-1}. Increases in light intensity based on a curve of optimum CO_2-fixation produced a final cell concentration of about 5.78 g/L. Both cultivation methods were carried out under ambient pressure at a temperature of 29°C with a superficial gas velocity of 2.4 m/h (U_G). Cells were grown on Beneck medium in a 1.0 L Bubble Column Photo bioreactor illuminated by a *Phillips* Halogen Lamp (20 W/12 V/50 Hz). The inlet gas had a carbon dioxide content of 10%.

Keywords: illumination, *Chlorella vulgaris* Buitenzorg, q_{CO2}, CO_2 fixation

INTRODUCTION

There have many attempts to decrease carbon dioxide content in the atmosphere through various means: physical, chemical, or biological treatments. One biological method that is able to reduce carbon dioxide is photosynthetic processing of micro algae [1]. Micro algae are *photolitotrophs* that perform oxygenic photosynthesis. Like higher plants, these organisms require radiant energy to eliminate the green house gas (GHG) CO_2 by using water as an electron donor. A significant contribution to photosynthesis is made by ribolose bisphosphate carboxylase/oxygenase (RubisCO). The reduced products of CO_2 serve as carbon sources for all biomaterials. In addition, micro algae are capable of absorbing a large amount of CO_2 by using an inducible CO_2 concentrating mechanism (CCM) [2].

A previous study on the micro algae *Synechococcus leopoliensis* IAM M6 cultivated on the modified Detmer medium (MDM) at 313 K showed an accumulation of external inorganic carbon ($\mathbf{C}_i$ pool; $\mathbf{C}_i = CO_2 + HCO_3^-$) to the extent of 0.128 $g \cdot L^{-1} \cdot h^{-1}$. The result is 5 to 9 times higher than the photosynthetic CO_2 fixation rate [3]. These characteristics suggest that the micro algae are potentially useful for study on the removal and utilization of CO_2 to minimize the accumulation of carbon dioxide emitted from industrial plant. For this reason, the utilization of photosynthetic microorganisms has become increasingly popular for CO_2-fixation and biomass production as a solution to the GHG problem. The species used for this purpose include *Anabaena cylindrica*, *Chlorella vulgaris*, *Dunaliella salina*, and *Spirulina platensis* [4-6].

Chlorella is widely known to contain potentially valuable substances; chlorophyll, CGF, beta-carotene, protein, and cellulose [7]. For its growth, *Chlorella* needs light energy and substrates. Light energy is an important factor for *Chlorella's* growth, as it is converted to chemical energy in the form of ATP to be used in photosynthesis, metabolism, growth, and cell division. CO_2 is fixated and used during dark reactions to produce carbon compounds [8]. Unfortunately, the levels of CO_2 fixation and biomass production achieved with constant light illumination are relatively low, because of the effect of self-shading. Furthermore, light energy needs are slightly increased during growth [9,10].

Based on previous works using *Chlorella, A. cylindrica*

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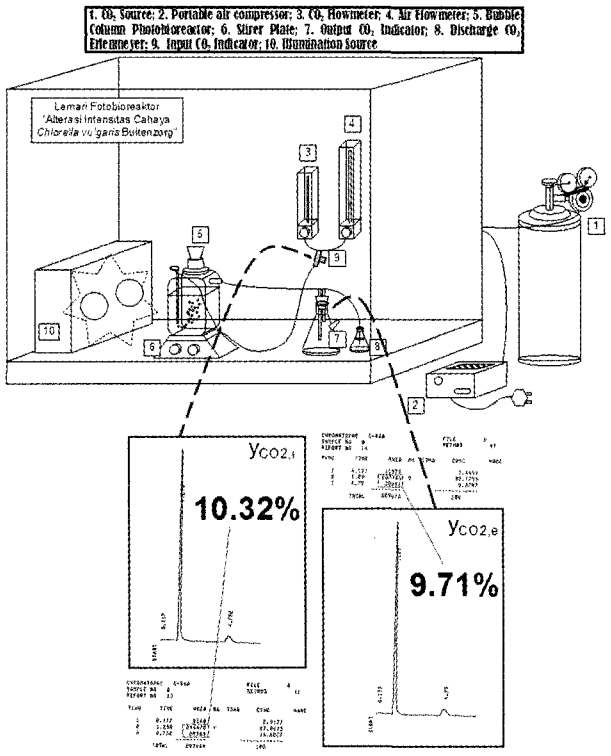

Fig. 1. Experimental scheme, and apparatus with raw data from Gas Chromatogram of percent CO_2 discharged from column ($y_{CO2,e}$) and percent CO_2 in air entering aeration inlet ($y_{CO2,i}$).

IAM M1, and *S. platensis* IAM M 135 [11-13], an effort to enhance CO_2 fixation and biomass production was implemented. This research used *C. vulgaris* Buitenzorg with increases in light intensity along with increases in culture biomass during the cellular growth period. The maximum levels of illumination for *Chlorella*'s maximum growth rate ($I\mu_{max,opt}$) and CO_2-fixation rate ($Iq_{CO2max,opt}$) were used in this alteration.

MATERIALS AND METHODS

C. vulgaris Buitenzorg strain was supplied by the Research Center of Fresh Water Fisheries, Department of Marine and Fisheries Depok, Indonesia. This strain was cultivated in a pyrex single bubble column photo bioreactor (1.0 L volume, 0.12 m side) containing Beneck medium. The research was divided into two stages. The first was to determine the light intensity for maximum specific growth rate and CO_2-fixation (q_{CO2}) of *C. vulgaris* Buitenzorg at several initial biomass concentrations. The results from this stage were used for the next stage, which was the alteration of light intensity. In the beginning, this photo bioreactor was illuminated from the side at 4.9 klux (77.4 μmol/[m^2·s]), which was increased up to 34.3 klux (542 μmol/[m^2·s]) in synchronization with the increases in biomass produced during the cultivation period. Temperature for these studies was set at 302 K, superficial gas velocity ($\boldsymbol{U_G}$) was 2.4 m/h, and inlet CO_2 composition ($y_{CO2,i}$) of enriched air was around 10.0%. Cultivation at a constant light illumination of 4.9 klux (77.4 μmol/[m^2·s]) ($\boldsymbol{I_i}$) was also performed as a control experiment. The adjustment of light intensity was performed by changing the distance between the side of photo bioreactor and the parallel banks of halogen lamps as shown in Fig. 1. Before the research experiment was begun, subcultures were prepared to avoid delays during the lag phase that usually occurs after cells are transferred from dense biomass cultures to fresh culture medium. The Chlorella subcultures were grown at 29°C with 3,000 lux of constant illumination (I_o) and were aerated at a rate of 2.4 m/h.

Measurement of OD_{680} of cell suspensions was performed for growth analysis. Calibration curves guided a relation where a unit of OD_{680} corresponded to a cell dry weight concentration of 0.72 g/L. CO_2 concentration of input and output gases ($y_{CO2,i}$, $y_{CO2,e}$) was analyzed by gas chromatography on a Shimadzu GC-8A GC-TCD. Light intensity of incident and transmitted light were measured by a Luxtron LX-103 Luxmeter and pH of all cultures was measured using a Hanna Model HI 8314 pH meter. All experiments were performed in triplicate to produce 3 replicates for each data point.

RESULTS AND DISCUSSION

In the first stage of the experiment, the strain of *C. vulgaris* Buitenzorg was cultivated to find the value of light intensity for obtaining maximum specific rates of growth (μ_{max}) and CO_2-fixation rate (q_{CO2}) for each initial biomass concentration. Seven (7) reaction mixtures were made with approximate initial biomass concentrations of ±0.71, ±1.43, ±2.89, ±6.39, ±10.0, ±13.7, and ±17.3 g/L. The range of illumination was between 4 klux (63.2 μmol/[m^2·s]) and 50 klux (790 μmol/[m^2·s]).

The results (Fig. 2) reveal that each variation gave a different light intensity for maximum μ value ($I\mu_{max,opt}$) and for maximum q_{CO2} value ($Iq_{CO2max,opt}$). This figure shows that any increase in biomass concentration is followed by an increase in the light intensity needed for maximum specific growth ($I\mu_{max,opt}$) and CO_2-fixation rates ($Iq_{CO2max,opt}$). These results also indicated that the photo bioreactor was a homogeneous system during the cultivation period. Furthermore, it was observed that the culture needed higher light intensities for cell growth. This result confirms previous research conducted by Hirata *et al.* [12], where increasing the intensity of illumination caused both the specific growth rate and the specific CO_2-fixation rate to increase to their maximum values. This figure was used as a guide for adjustments in light intensity (Fig. 3).

Illumination, $\boldsymbol{I_i}$ was initially set at 4.9 klux (77.4 μmol/[m^2·s]) and adjusted every 4 h to maintain the maximum specific growth at a high rate in accordance with the results from the first stage of the experiment. The $\boldsymbol{I_i}$ values obtained from this report were the optimum conditions at the point of alteration of light intensity, that was indicated to a possible maximum value of specific

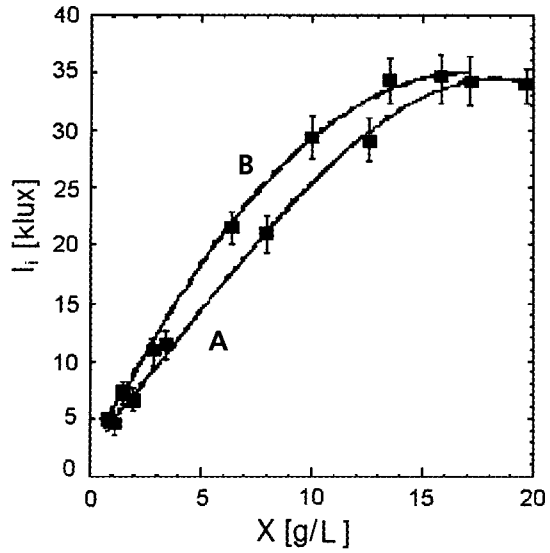

Fig. 2. Light intensity alteration-based curves [Light intensity *versus* culture biomass concentration]. (A) q_{CO2max}-based curve; (B) μ_{max}-based curve.

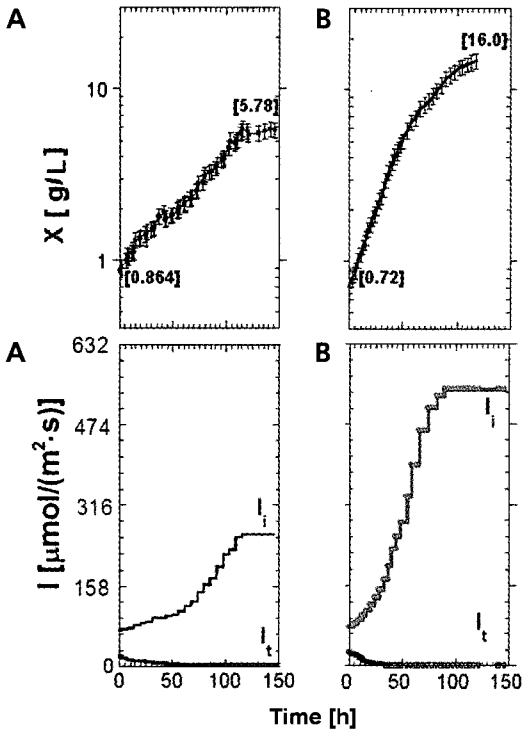

Fig. 3. Illustration of light intensity alteration and it's effect on biomass production. (A) q_{CO2max}-based curve; (B) μ_{max}-based curve.

growth rate and specific CO_2 fixation rate.

The experimental result was shown in Fig. 3. The figure illustrates a comparison of effects of the alteration of light intensity between q_{CO2max} base and μ_{max} base to the produced final biomass concentration (X_f) during cultivation period. It was revealed that the final biomass concentration produced in μ_{max}-based alteration of illumination is higher than that from q_{CO2max}-based alteration of illumination. During the 118-hour cultivation period, increases in light intensity based on μ_{max} produced a final cell concentration of about 16.0 g/L. However, illumination increases based on growth only produced a final cell concentration of about 5.78 g/L. This was despite the fact that the latter had a longer cultivation period, which was about 146 h.

Table 1. The acquired light energy utility for biomass production (E_x) and efficiency of light energy utility for biomass production (η_{bp})

Light illumination	E_x (J/kg)	η_{bp} (%)
Alteration (μ_{max} base)	44.3	0.11
Alteration (q_{CO2max} base)	55.5	0.13

The above figure notation of I_i stands for the photosynthetic photon flux density (PPFD) incident light intensity at the inner front surface of the bioreactor and the notation of I_t stands for the transmitted light intensity at inner back surface of the bioreactor. The comparison of calculated light energy utility for biomass production ($\mathbf{E_x}$) based on result of I_i and I_t in the both experimentals showed that, cultivation with light intensification based on q_{CO2max} showed more efficient results (Table 1).

The value of E_x in this experiment lies between the two methods used in previous experiments. It means that the energy consumed for cultivation is larger than the increase in illumination needed for maximum growth. Nevertheless, the conversion of light energy to biomass produced gives better results, as shown by higher efficiency (η_{bp}). Based on a significance multiplicity of light energy flux for photosynthesis, it could be understood that the conversion efficiency value of a q_{CO2max}-based illumination mode is higher than the other illumination modes.

During micro algae's photosynthesis, carbon dioxide from the air and other carbon sources entered the culture media to become CO_2, H_2CO_3, HCO_3, and H^+. The carbon dioxide and bicarbonates went into the cell and participated in several reactions by using the energy of the sun as an illumination source (Fig. 4).

Some of the carbon dioxide available is transformed into glucose through the TCA cycle and subsequently becomes cell materials through enzymatic reactions. The rest of the carbon dioxide enters a vacuole that functions as the $\mathbf{C_i}$ pool (accumulation pool of external inorganic carbon cellular). The carbon transfer rate of *C. vulgaris* Buitenzorg is determined by these phenomena. During microbial growth, the density of micro algae causes self-shading of light, where micro algae at the front of the photo bioreactor are covering those behind them [2]. To overcome this barrier to photosynthetic capability, higher light intensity is needed to increase cell metabolism and to maintain the carbon transfer rate equilibrium. These are the main reasons why changes in light intensity were required in our research.

The result of *Chlorella*'s CO_2 fixation capability in this research is shown in Fig. 5. This figure shows that the enhancement of illumination based on maximum q_{CO2}

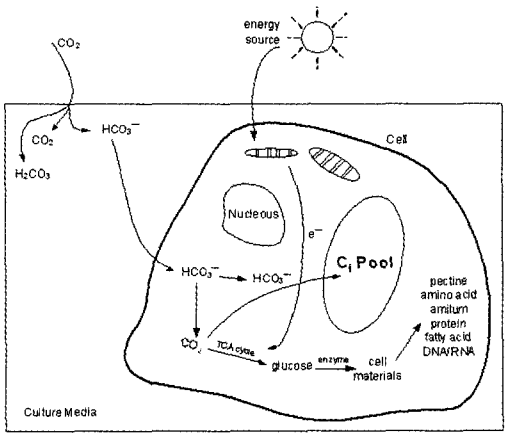

Fig. 4. Illustration of cell metabolism [3].

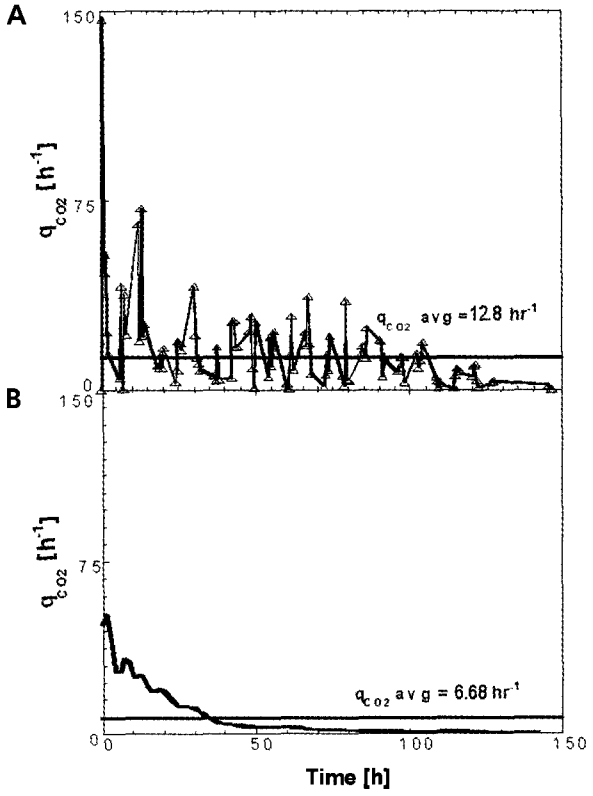

Fig. 5. Comparison of q_{CO2} values; (A) q_{CO2max} base; (B) μ_{max} base.

values produced a higher average q_{CO2} than the light intensity increases based on maximum biomass production. The experiment based on maximum q_{CO2} values produced an average q_{CO2} value of about 12.8 h^{-1}, while the other one only produced an average value of 6.68 h^{-1}.

CONCLUSION

The alteration done by periodically raising light illumination, starting from 4.9 Klux (77.4 μmol/[m^2·s]) for the cell density of 1,000,000 cell/cm^3 (equal to 0.084 g/L). According to the previous results (Fig. 3), there was a significant difference in the biomass produced with q_{CO2max}-based illumination when compared with other methods. This was caused by the difference in the optimum light intensity used in both experiments. The optimum intensity for maximum q_{CO2} value was in relatively narrow range. It can be concluded that the light intensity required for maximum q_{CO2} values is not the same as the light intensity needed for maximum biomass production.

NOMENCLATURE

E_x	The acquired light energy utility for biomass production
I_i	Incident light intensity at the inner front surface of the bioreactor [klux]
$I\mu_{max,opt}$	Incident light intensity for maximum μ_{max} value [klux]
$Iq_{CO2max,opt}$	Incident light intensity for maximum $q_{CO2,max}$ value [klux]
I_t	Transmitted light intensity at inner back surface of the bioreactor [klux]
M_{CO2}	Molecular weight of CO_2 [g/mol]
μ	Incident specific growth rate [h^{-1}] $\mu = \frac{1}{X}\cdot\frac{dX}{dt}$
μ_{max}	Maximum specific growth rate [h^{-1}] $\mu_{max} = \lim_{t\to 0}\frac{1}{X}\cdot\frac{dX}{dt}$
η_{bp}	Efficiency of light energy utility for biomass production [–]
PPFD	Photosynthetic photon flux density [μmol/m^2·s]]
q_{CO2}	Incident CO_2-fixation rate [h^{-1}] $q_{CO_2} = Q_G\cdot\frac{y_{CO_2,i}-y_{CO_2,e}}{V\cdot R\cdot T}\cdot\frac{M_{CO_2}\cdot P_T}{X}$
q_{CO2max}	Maximum CO_2-fixation rate [h^{-1}]
Q_G	Volumetric gas flow rate measured at gas inlet [L/h]
P_T	Ambient pressure [Pa]
R	Universal gas constant [= 8.31·10^6 Pa·L·K^{-1}·mol^{-1}]
t	Time [h]
T	Culture medium temperature [K]
U_G	Superficial gas flow rate measured at gas inlet [m/h]
V	Volume of culture medium [L]
X	Cultural biomass concentration [g/L]
$y_{CO2,i}$	CO_2 concentration of input gas [%]
$y_{CO2,e}$	CO_2 concentration of output gas [%]

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