BIOMASS PRODUCTION *Chlorella vulgaris* BUITENZORG USING SERIES OF BUBBLE COLUMN PHOTO BIOREACTOR WITH A PERIODIC ILLUMINATION

Anondho Wijanarko, Dianursanti, Muryanto, Josia Simanjuntak, Praswasti Pembangun Dyah Kencana Wulan, Heri Hermansyah, Misri Gozan, and Roekmijati Widaningroem Soemantojo

Department of Chemical Engineering, Faculty of Engineering, University of Indonesia, Depok 16424, Indonesia

E-mail :anondho@che.ui.edu

Abstract

Chlorella vulgaris Buitenzorg cultivation using three bubble column photo bioreactors arranged in series with a volume of 200 mL for 130 hours shows an increase of biomass production of *Chlorella vulgaris* Buitenzorg up to 1.20 times and a decrease of the ability of CO_2 fixation compared to single reactor at a periodic sun illumination cycle. The operation conditions on cultivation are as following: T, 29.0^oC; P,1 atm.; U_G, 2.40 m/h; CO_2, 10%; Benneck medium; and illumination source by Phillip Halogen Lamp 20W /12V/ 50Hz. Other research parameters such as microbial carbon dioxide transferred rate (qco_2), CO_2 transferred rate (CTR), energy consumption for cellular formation (E_x), and cultural bicarbonate species concentration [HCO_3] also give better results on series of reactor.

Keywords: Chlorella vulgaris Buitenzorg, *sunlight, periodic illumination, photo bioreactor, series configuration*

1. Introduction

Global warming has become one of the most serious environment problems. The main cause of this is because of the increasing of CO_2 level in the atmosphere. In recent years, many attempts have been done to reduce the quantity of CO_2 in the atmosphere. Studies on photosynthesis, CO_2 fixation and utilization of micro algae biomass has been carried out. Similar to another *Chlorella* strain, *Chlorella vulgaris* Buitenzorg is known widely of its high valued potential substances such as chlorophyll, CGF, beta-carotene, and protein, and it can be used as potential biomass albeit the function of CO_2 fixation [1-4].

Previous research objectives are to increase CO_2 fixation and the amount of biomass *Chlorella vulgaris* Buitenzorg with various types of illumination (e.g. continuous illumination, cycle illumination, and alteration illumination) at a single photo bioreactor [2-6]. This research uses several photo bioreactors configured in series to increase the amount of biomass and CO_2 fixation by using daily cycle periodic illumination. Periodic illumination is used to accommodate the real condition of cultivation for open purpose application.

2. Materials and Methods

Chlorella vulgaris Buitenzorg is taken from Depok Research Center of Fresh Water Fishery that was grown in Benneck medium. This strain grows in series of 0.20 dm^3 bubble column photo bioreactor. The operation conditions on cultivation was defined as following, Temperature (T) set at 29.0 ^oC, Pressure (P) was set at ambient temperature (1 atm.), superficial gas velocity (U_G) set at 2.40 m/h and CO_2 concentration (y_{CO2i}) in sparged bubble air set at 10.0%. These photo bioreactors are arranged in series and illuminated by *Philips Halogen* lamp 20W/12V/50Hz.

Photo bioreactors run with varied light intensity given accordingly to the daily light intensity of sunlight on serial bubble column photo bioreactors. This data will be compared to the single reactor cultivation at the same illumination. The experimental apparatus used in the experiment is shown on **Fig. 1**.

Data obtained from this experiment are OD_{680} (Optical density that was measured using spectrophotometer at 680 nm and correlated with biomass concentration X), y_{CO2} using GC-TCD (*Gas Chromatography – Thermal Conductivity Detector*) pH using pH meter, and I_T (transmitted light intensity that was measured behind of photo bioreactor) using Lux-meter.

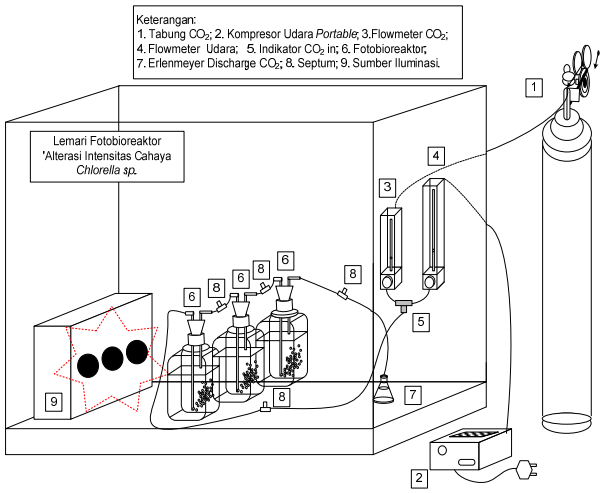

Fig. 1. Experimental Apparatus

Initial inoculums for this experiment are 4 500 000 sel/cm^3 that was equivalent to 4.17 g/dm^3 of dry biomass concentration. This is the biomass cell concentration that tend optimum growth rate at average daily cycle intensity (39.8 W/m^2). Before cultivation, pre-culture is done by sparging the air into the reactor. Light with intensity 2.95 W/m^2 is also given in order to pass the lag phase of *Chlorella vulgaris Buitenzorg*'s growth.

3. Result and Discussion

The biomass production using both of single and serial photo bioreactors is shown on **Fig. 2.** Comparison the experimental result in both of results, indicated that microbial growth in serial of photo bioreactors higher than in single one. In the same initial dry biomass concentration of 4.17 g/dm^3, it is seen that in each serial mode photo bioreactor, the slope of growth curve of *Chlorella vulgaris* Buitenzorg that was indicated its growth rate, is sharper than in single one. This case was already happen caused of the mixing phenomenon of nutrients and culture cells are better in serial photo bioreactors [7-9].

Above figure showed that the curve of biomass production at daily cycle increase in light condition and the curve will be flat and descend at the dark cycle. In the light condition, *Chlorella vulgaris Buitenzorg* receive light for photosynthesis to produce carbohydrates for its growth. Meanwhile, at dark condition, *Chlorella vulgaris Buitenzorg* can not receive light and used its own carbohydrates to maintain their live. Utilization of its carbon source caused biomass concentration become constant or slightly decreased [5].

Fig. 3 and **4** shows the growth rate of *Chlorella vulgaris* Buitenzorg at single reactor and series of multiple reactors. The biomass growth rate in each reactor at serial mode tends higher than at single mode. The average microbial growth rate in first reactor is around 1.28 times of result in single reactor. The average growth rates of second and third reactor tend around 1.25 and 1.10 times.

The calculation of [HCO_3^-] is done to figure out the amount of provided bicarbonate ions so that could be consumed during its microbial growth. [HCO_3^-] is calculated from the changing the measured culture pH using the approximation of Henry's law and the *Hendersen-Hasselbac*h correlation, which was shown in following equation.

$$[HCO_3] = \left(\frac{K_{CO_2,0}}{H_{CO_2,0}}\right)\left(\frac{y_{CO_2 i} P_T}{10^{-pH}}\right) \cdot \left(\frac{\exp[A_k(1-To/T)+B_k \ln(T/To)+C_k(T/To-1)]}{\exp[A_H(1-To/T)+B_H \ln(T/To)+C_H(T/To-1)]}\right) \quad (1)$$

Here, the A_k, B_k, C_k defined as thermodynamic parameters of carbon dioxide equilibrium constant and A_H, B_H, C_H defined as thermodynamic parameters of carbon dioxide Henry constant. y_{CO2i} is CO_2

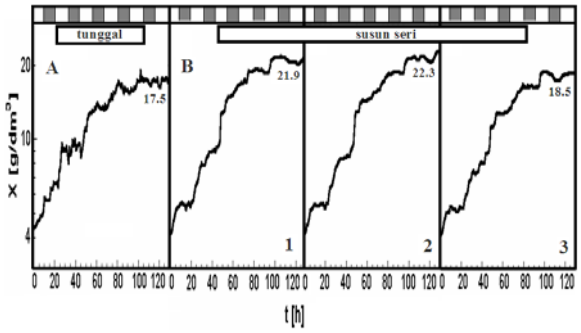

Fig. 2. Biomass Production (*X*) in both of single (tunggal) and serial (susun tunggal) photo bioreactors

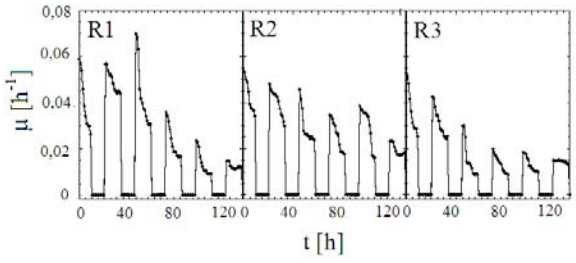

Fig. 3. Growth rate (μ) in series photo bioreactor

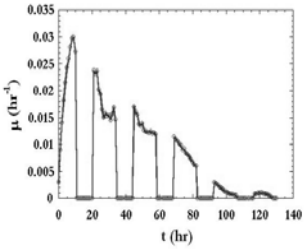

Fig 4. Specific growth rate (μ) in single photo bioreactor

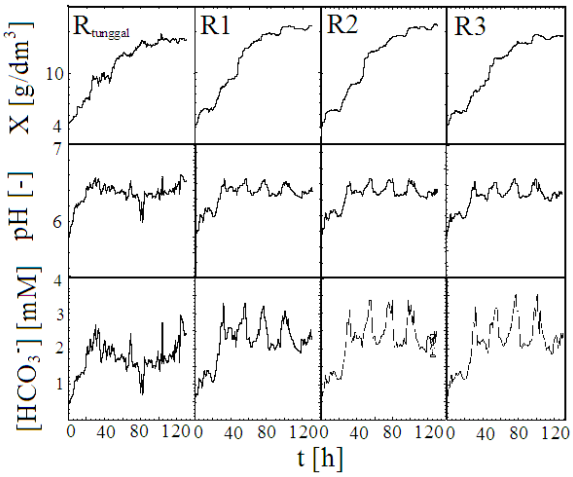

Fig. 5. pH and [HCO_3^-] in single (*tunggal*) and serial photo bioreactors

concentration in sparged bubble air, P_T is incident pressure, T is incident temperature, $K_{CO2,o}$ and $H_{CO2,o}$ are equilibrium and Henry constant at standard condition.

The data of ion bicarbonate using pH on culture which is obtained from the experiment can be seen in **Fig. 5**. The concentration of bicarbonate ion increases due to the increase of the pH of the medium, then the value of [HCO_3^-] will be higher corresponding to value of pH. **Fig. 5** shows that the maximum [HCO_3^-] in single reactor is 2.94 mM and the average of [HCO_3^-] in multiple reactor series is 3.39 mM. Thus, the series of reactor can enhance the concentration of bicarbonate up to 1.15 times.

The calculated *carbon dioxide transferred rate* (CTR) shown the amount of CO_2 gas that transferred in a volume of medium that needed for cell metabolism in a certain time period. This CTR value was calculated from the difference between photo bioreactor inlet and outlet CO_2 concentration (Δy_{CO2}) that was caused due to the transfer of CO_2 from air sparged bubble into medium bulk and consumption of CO_2 as substrate by *Chlorella vulgaris* Buitenzorg, which was shown in following equation.

$$CTR = \alpha_{CO_2} \cdot \Delta y_{CO_2} \qquad (2)$$

Here, α_{CO2} defined as CO_2 fixation constant that was consisted superficial gas velocity of air sparged bubble and its ambient conditions.

The calculation of microbial CO_2 transferred rate that was also defined as cultural CO_2 fixation rate (q_{CO2}) is done to figure out the microbial ability to fixate CO_2 together with its biological activities to grow up their colony in culture media. This q_{CO2} value was also calculated from the difference between photo bioreactor inlet and outlet CO_2 concentration (Δy_{CO2}), which was shown in following equation.

$$q_{CO_2} = \alpha_{CO_2} \cdot \frac{\Delta y_{CO_2}}{X} \qquad (3)$$

The calculated data of CTR and q_{CO2} using difference between photo bioreactor inlet and outlet CO_2 concentration of bubble sparged air in culture and its incident biomass concentration which is obtained from the experiment, can be seen in **Fig. 6** and **7**.

At the beginning of cultivation, the average value of CTR increases due to CO_2 gas transfer or *Chlorella* consumption for cell metabolism. The increasing in growth rate is caused by the increasing of CO_2 fixation increase. This is proportional with the energy consumption of bicarbonate ion by Chlorella vulgaris Buitenzorg CTR average (*CTR rata*^{2}) value in each reactor are 22.1 g/dm^{3}h, 12.9 g/dm^{3}h, and 11.5 g/dm^{3}h.

In this experiment, q_{CO2} value can be obtained by dividing CTR with the dry biomass concentration of cell. The increasing growth *Chlorella vulgaris* Buitenzorg in photo bioreactor causes the decreasing of q_{CO2}. This degradation is caused by imbalance between the increasing of cell during the period of cultivation and the fixated level of CO_2 concentration. The average value of q_{CO2} in each reactor (q_{CO2} *rata*^{2}) is 2.43 h^{-1}, 1.43 h^{-1}, and 1.45 h^{-1}. Fig. 7 shows single reactor has lower value of q_{CO2} compared to multiple reactor series. This means each biomass product in a serial system of bubble column photo bioreactors gives a better ability for CO_2 fixation. The average value of CTR and q_{CO2} in single reactors are 18.4 g/dm^{3}h and 1.95 h^{-1}. The decrease of microbial CO_2 fixation ability in this serial reactor with periodic illumination is hardly different to the result with alteration of illumination [7-9]. Compare to the result in single reactor, It was predicted that individual cellular growth ability after dark period using CO_2 more efficient [6].

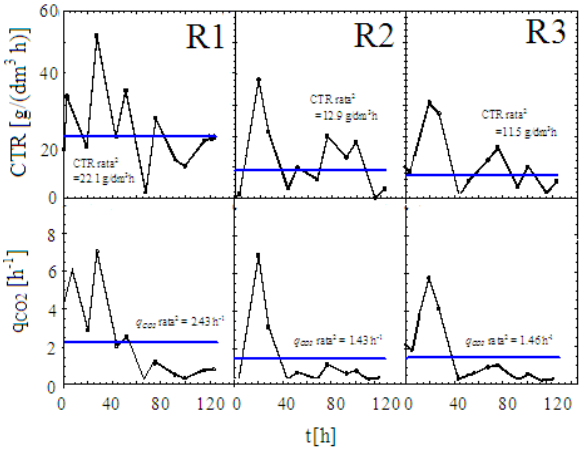

Fig. 6. CTR and q_{CO2} in serial photo bioreactors

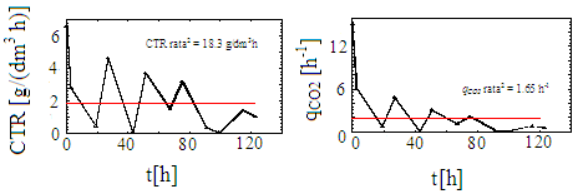

Fig. 7. CTR and q_{CO2} in single photo bioreactor

Table 1. Calculated biomass formation energy of *Chlorella vulgaris Buitenzorg*

Type Reactor		E_x (kJ/g)
Multiple reactor series	1	0.14
	2	0.16
	3	0.17
Single Reactor		0.02

The amount of energy used for the *Chlorella vulgaris* Buitenzorg's biomass formation that was calculated by equation 4, is shown on Table 1.

$$E_X = \frac{\int_o^t I_T \cdot dt}{\Delta X \cdot s} \qquad (4)$$

Here ***s*** was defined as light path length across culture medium.

The average value of energy provided in multiple reactor series is greater than the energy in single reactor because the effect of smaller self-shielding breakeven reactor so that the distribution of light becomes better. Therefore, multiple reactor series requires larger energy for the growth process of *Chlorella vulgaris* Buitenzorg, These value more higher than result of *Anabaena cylindrica* Lemmerman on continuous illumination and also *Chlorella vulgaris* Buitenzorg on both of continuous and alteration of illumination [7-9]. It predicted that individual cellular can adapt the gradual increasing of illumination than in flip-flop illumination, which also take more time to repair and produce internal photosynthetic apparatus for light captured during dark period [5,6].

4. Conclusion

Multiple reactor series can increase the biomass production (X) up to 1.2 times compared to the biomass production in single reactor.

The ability of *Chlorella vulgaris* Buitenzorg for CO_2 fixation in serial photo bioreactors tend a decreasing compare to result in single reactor.

The energy used in the cultivation of *Chlorella vulgaris* Buitenzorg in multiple reactor series is greater than single reactor.

References

[1] S. Wirosaputro, Chlorella Untuk Kesehatan Global, Gadjah Mada University Press, Yogyakarta, 2002.
[2] A. Wijanarko, Dianursanti, Heidi, R.W. Soemantojo, K. Ohtaguchi International Journal for Algae 8 1 (2006) 53-60.
[3] A. Wijanarko, Dianursanti, M. Gozan, S.M.K. Andika, P. Widiastuti, H. Hermansyah, A. B. Witarto, K. Asami, R.W. Soemantojo, K. Ohtaguchi, S.K. Song, Biotechnology and Bioprocess Engineering, 11 (2006) 484-488.
[4] A. Wijanarko, S.M.K. Andika, Dianursanti, A.B. Witarto, K. Asami, K. Ohtaguchi, R.W. Soemantojo, 8th International Conference on Carbon Dioxide Utilization, Oslo, Norway (2005) 48-49.
[5] J.C. Ogbonna, H. Yada, H. Tanaka, Journal Fermentation and Bioengineering, vol. 80 No. 7 (1995) 259-264.
[6] A. Wijanarko, Dianursanti, A.B. Witarto, R.W. Soemantojo, J. Makara, vol. 8, No. 2 (2004) 35-44
[7] A. Wijanarko, K. Ohtaguchi, Studies in Surface Science and Catalysis, 153 (2004) 461-468.
[8] A.Wijanarko, Dianursanti, M. Gozan, Regional Symposium on Chemical Engineering, Singapore, (2006) 249-250.
[9] A.Wijanarko, Dianursanti, M.A. Sujarwo, M. Gozan, First Indonesia-Korea Biotechnology Symposium, Jakarta, Indonesia (2007) 1-6.