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EFFECT OF PHOTOPERIODICITY ON CO_2 FIXATION BY *Chlorella vulgaris* Buitenzorg IN BUBBLE COLUMN PHOTOBIOREACTOR FOR FOOD SUPPLEMENT PRODUCTION

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Abstract

To reduce the level of CO_2 content in air, effort on converting CO_2 to useful products is required. One of the alternatives includes CO_2 fixation to produce biomass using *Chlorella vulgaris* Buitenzorg. *Chlorella vulgaris* Buitenzorg is applied for production of food supplement. *Chlorella vulgaris Buitenzorg* is also easy to handle due to its superior adaptation. Currently, *Chlorella vulgaris Buitenzorg* has been analyzed by some experts for its cellular composition, its ability to produce high quality biomass and the content of essential nutrition. A series of experiments was conducted by culturing *Chlorella vulgaris* Buitenzorg using Beneck medium in bubbling column photobioreactor. The main variation in this experiment was photoperiodicity, where growth of *Chlorella vulgaris* Buitenzorg was examined during photoperiodicity condition. The difference between CO_2 gas concentration of inlet and outlet of the reactor during operational period, was compared to the same experiment under continuous illumination. Under photoperiodicity of 8 and 9 h/d, the culture cell densities (N) were approximately 40 % higher than under continuous illumination. Final biomass density of *Chlorella vulgaris* Buitenzorg at 9 h/d illumination was 1.43 g/dm^3, around 46% higher than under continuous illumination. Specific carbon dioxide transfer rate (q_{CO2}) in photoperiodicity was 50-80% higher than under continuous illumination. These experiments showed that photoperiodicity affects the growth of *Chlorella vulgaris* Buitenzorg The specific growth rate (μ) by photoperiodicity was higher than that by continuous ilumination while the growth period was two times longer. Based on the experiments, it can be concluded that photoperiodicity might save light energy consumption. The prediction of kinetic model under continuous illumination as well as under photoperiodicity illumination showed that Haldane model became the fitted kinetic model.

Keywords: photoperiodicity, Chlorella vulgaris Buitenzorg, food supplement, CO_2 fixation, Haldane model.

1. Introduction

In the last decade global emission of green house gas of carbon dioxide from fossil fuel combustion increased 8.1%, that was around 0.6 billion Giga grams (Gg). The global atmospheric concentration of this green house gas has increased by 31% since 1750 and a level of 366.7 ppm in 1998. The increasing rate of atmospheric carbon dioxide concentration has been around 1.5 ppm per year over the past two decades [1].

Global scale carbon dioxide fixation naturally occurs during photosynthesis in green plants. However, the efficiency of solar energy conversion in plant production under optimal growth conditions is only 5 to 6%. Under field conditions, even high-yielding crops such as maize, bulrush millet or sugarcane, convert solar energy into plant material with maximal efficiency of 1 to 2%. Most major crops and forest achieve much lower efficiencies. The global average efficiency has been estimated as 0.15%. There is thus considerable interest in other bioconversion pathway for carbon dioxide fixation. One of these is that performed by the micro algae [2].

Study on assimilation of carbon dioxide during photosynthetic microbial growth of micro algae, cellular carbon was formed from CO_2 through the ribulose biphosphate pathway and indicated that fixation of carbon dioxide also followed the Calvin cycle that occurs in a direct synthesis to carbohydrate via glucose production [2].

Photosynthesis in general perception is defined as a process of building new chemical compounds using the energy of light. The rate and the efficiency of photosynthesis on how photosynthetic organisms can grow is a major limiting factor that further limits the biomass production of all agriculture and food production. Photosynthesis is carried out in sub-cellular objects called chloroplasts, which have membranes consisting of light capturing parts of photosynthesis. Light reactions and dark reactions occures during photosynthesis. During light reactions the energy of light is captured. During dark reactions the energy is used to capture carbon dioxide from the air and link it into growing sugar molecules [2].

The light reactions happen in 2 photo-systems. In the first system, the light is captured by an antenna complex, a large array of protein molecules that is almost 100% effective in capturing the photons falling on them. The energy is then transferred to electrochemical reactions that generated ATP, or is used to split water into hydroxide, which forms oxygen and hydrogen gases, which is used to reduce cytrochrome proteins. The second system boosts the energy of the electrons involved in this process to a sufficient high level energy in two steps, due to the first system on its own could not generate enough energy to split water. The captured energy is accumulated as reduced NADH and ATP, which is used in the dark reactions. The dark reactions are centered around the reaction of carbon dioxide with a sugar called ribulose diphospate, producing two three-carbon sugar molecules. It is catalyzed by ribulose-1,3-diphosphate carboxylase/ oxygenase, always referred to as RUBISCO [2].

A whole series of other reactions then convert the products into other sugars, and thence into plant. RUBISCO is the most common protein in earth, and it is used in the basic photosynthesis process in green plants such as micro algae and other simple plants and is called the C3 process, because it centers around three-carbon sugars. Photosynthesis builds sugars from CO_2 and light, generating oxygen as a side product. Another process is called photorespiration which that exactly the opposite and unfortunately uses the same enzyme, RUBISCO [2].

In tropical countries, photorespiration almost equal to photosynthesis, and the higher plants adapted to these conditions, use a more complex scheme called "C4". In this process, carbon dioxide is carried on a chemical carrier molecule to the chloroplasts, where it is concentrated before used in photosynthesis, thus RUBISCO rich in CO_2. The difference between "C3" and C4 photosynthesis is not just a matter of different enzymes. The leaves of C4 plants are arranged so that the CO_2-capturing enzymes are on the outside and the CO_2-releasing enzymes are inside next to the chloroplasts [2].

As a note of this explanation, the other essential products like β carotene and other type of pigments, γ linolenic acid and other poly un-saturated fatty acids, essential amino acid and so on, were performed with another complex and sequel cellular bioprocess pathway [2].

Above explanation clarified a general process of light reaction and dark reaction of photosynthesis of plants. It was also need more clarification around the advantage of dark period on growth of micro algae. Following explanation was correlated with proposed experimental aim of periodic illumination of CO_2 removal and biomass production ability of micro algae [3].

Refer to the previous work in *Prochlorococcus* sp., as one of species of prokaryotic - micro algae - and was identified as a smallest phytoplantonic organisms [3,4,5], it was examined the effect of illumination on cell growth cycle. This work concluded that during alteration of high values to low values of illumination, caused a decrease of cellular growth rate happened that was indicated by a relatively constant values of cell concentration during dark period. But an increasing general cell metabolism and cellular "*chlorophyll a"* formation was still occured.

A very small increase of cell concentration, that happened during dark period, caused only a few complete process of cell growth formation which was supported by a fact of a decrease value of number of cells that synthesized DNA. The cells already prepares for mitosis which was called as cell in "S" and "G_2" phases of cell cycles. Based on this observation it was concluded that during dark period of alteration of high light illumination to low light illumination, the cells is at - *no cell divide step* - [3].

On the contrary, during alteration of dark period to light period, that was conducted by the alteration of low light illumination to high light illumination, microbial cell concentration increased quickly that was espoused also by rapid increase of percentage of S and G_2 phase of cell cycle, which was defined as the occurrence of cell dividing [3].

Similar to above explanation, an opposite result was also happened in the case of the alteration of low light illumination to high light illumination, that was caused by a decrease value of DNA and "*chlorophyll a*" content of the cells [4,5].

Results of studies of interaction between light intensity on photosynthesis of *Oscillatoria agardhii* [6], also supported above explanation which concluded that an

increase of light illumination caused a decrease of cellular "*chlorophyll a"*.

Risk reducing strategy, which actually drives carbon sequestration to release people from the threat of climate change, should be taken as a result of explosive increase of human population and rapid growth of the usage of energy and other resources. The strategy includes efforts to formulate technology regarding new forms of energy production processes that embody an ecological perspective, such as conversion of carbon dioxide by micro algae. This photosynthetic process of micro algae produce extremely higher carbon dioxide removal than that of higher plants.

Culture cyanobacterium *Synechococcus leopoliensis*, *Anabaena cylindrica* Lemmerman, *Spirulina platensis*, *Oscillatoria* sp. green algae *Chlamidomonas* Spp. Ehrenberg, *Scenedesmus* sp and domestical microorganisms *Chlamidomonas* sp. NBP and *Chlorella vulgaris* Buitenzorg, had been investigated for high carbon dioxide fixation in aerated liquid in bioreactor. These strains are usually rich in starch and other carbohydrates of photosynthetic origin which can be transformed into bio-fuel ethanol and hydrogen as partial substitute for non renewable resources [7] and also rich in other essential product like β carotene, γ linolenic acid and other poly unsaturated fatty acid, that are safe to be consumed as food and supplement goods.

The prominent advantages of this starch production method over conventional starch production which has been conducted in the plantation of corn, wheat, barley, rice and other cereals, are the easiness of process control, the lower cost of energy and the man power required for plantation, cropping, harvesting and transportation.

To design the bio-production unit in the application of outdoor culture for CO_2 removal and using sunlight as energy source, especially in tropical region, it is necessary to examine photoperiodicity condition during the growth of domestic micro algae. Furthermore this experiment would be continued to outdoor for large scale application. The objectives of this experiment are :

1. To investigate the effect of photoperiodicity on the growth of micro algae and CO_2 fixation in Benneck medium where the pH of culture were uncontrolled.
2. To predict the kinetic model of micro algae growth under photoperiodicity of "*illumination hours per day*" (τ_i) where the pH of culture were uncontrolled.

2. Methods

This experimental works was modeled in a laboratory scale. It was performed with periodic illumination of 6, 7, 8, 9 h/d, which represents daily illumination of the sun in tropical region. A domestic unicellular green algae, identified as *Chlorella vulgaris* Buitenzorg, was supplied from micro algae culture collection of Fresh Fishery Service of Depok City. This micro algae was grown in the 500 cm^3 bubble column photo bioreactor (0.08 x 0.08 m^2 cross sectional area, pyrex glass made) containing synthetic media based from Benneck at ambient temperature (Figure 1).

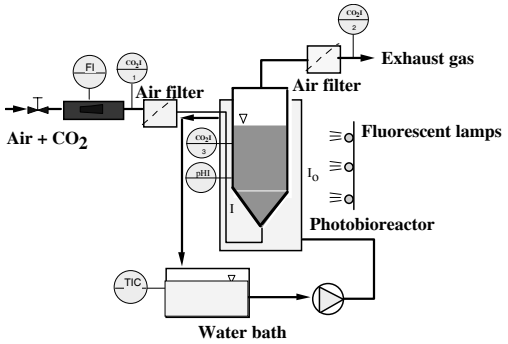

Figure 1. Experimental apparatus

The culture was aerated by carbon dioxide enriched air (5% CO_2) with superficial gas velocity U_G of 2.4 m/h. This vessel was illuminated at 6.0 klx (I_o) by parallel banks of fluorescent lamps (FPL 27 EX-N, National Electronics Co., Japan) under photoperiodicity and continuous illumination. In this case, the average value of "*illumination hours per day*" (τ_i) was determined in the range of 6.0 - 9.0 h/d. during the dark period the sides of vessel were covered by aluminum foil. Initial microbial pH of was controlled at 7.8. Inocula for above growth experiment were collected from the pre-culture vessel (0.5 dm^3 oblong flat flask) which was cultivated in Benneck medium, illuminated at 1.0 klx and aerated at 1 vvm superficial gas velocity by plain air for 72 h. Here 1 vvm (one volume of medium aerated with one volume per minute of plain air).

Growth analysis was done by measuring microscopic cell counting of cell suspension. Pre experimental observation resulted that cellular dry weight conversion was $7.72.10^{-10}$ g/cell. Monitoring the incident and transmitted light intensity was carried out by Luxmeter. The content of carbon dioxide in bubbling air before and after passing the culture medium was measured by Gas Chromatography analysis. pH value of culture medium was monitored by pH meter.

3. Results and Discussion

The rate of photosynthesis process is highly affected by its ability in absorbing light. This photosynthesis rate is proportional to the cell growth rate of *Chlorella vulgaris* Buitenzorg Maximum photosynthesis rate was obtained around wavelength of 450 nm and 680 nm. It had been

proven that growth of *Chlorella vulgaris* Buitenzorg in photo bioreactor using wavelength of 450 nm (blue light) is better compared to that using other lights having wavelength smaller than 450 nm (Figure 2). Red light illumination (700 nm), produced a growth rate nearly equal to that of blue light, while by using white light, its growth rate is between that of yellow light and red light. It is possible, because white light contains all types of lights, so that the growth of micro algae is the mean of every wavelength's growth. Illumination using UV light indicates a decrease in cell amount and the color of some micro algae turned white. In this case, photo bleaching was happened. This susceptibility to photo inhibition is caused by the blockage of chloroplast-encoded protein synthesis, and a severe damage occurs in photo-system (PS) II reaction center of micro algae *chlorophyll a* [8-10].

According to the reference [11], the largest composition of wavelength intensity received by the surface of the earth is acquired in visible light range, which can be seen by human- naked eyes (Figure 3). Most of UV and IR light are scattered and reflected by the atmosphere. If the condition of the ozone layer remains stable, the proportion of UV light is predicted to be not greater than 2%. According to the total curve area, UV light has the smallest intensity proportion; it is only around 1.94%. The biggest proportion is visible light wavelength ranging between 400 nm – 800 nm and followed by IR light. The yellow and green light have proportion almost equal, only approximately 15% each. The Blue, Red and Infra Red are about 23.6%, 24.8%, 19.9% respectively [11]. This is the reason why UV-light effect that damaged cellular *chlorophyll a* and destroyed micro algae in the open air, is not significant. While the effect of visible light, especially red and blue, is very high, due to their bigger proportion in the sunlight. Red and blue are the most important lights for photosynthesis process.

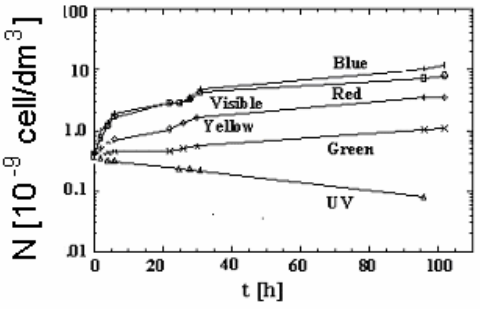

Figure 2. The Growth of *Chlorella sp* in wavelenght variation

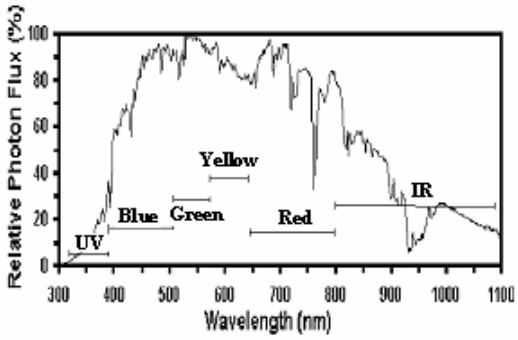

Figure 3. Earth surface sunlight spectrums composition in 12:00 noon [11].

In photosynthesis process, *Chlorella* sp needs light energy to alter CO_2 into inorganic substances such as carbohydrates, that will be used as source of food. Visible light illumination is important for *Chlorella vulgaris* Buitenzorg to maintain cell cultivation. For open pond application purpose, this research was carried out under cycle of light-dark illumination (photoperiodicity). This result was compared to the result of similar research under continuous illumination. The variation of photoperiodesity was 6, 7, 8, 9 light illumination hours per day. The operating condition of this research was set at tropical ambient temperature and pressure (25 °C, 1 atm.) and the number of initial cells was ± 200.000 cells/cm^3.

From the curve of cell-growth curve results (Figure 4), it is clear that in the early cultivation stage, the growth under continuous illumination was faster than the growth in light-dark cycle illumination. Final biomass density of *Chlorella vulgaris* Buitenzorg at 9 h/d illumination was 1.43 g/dm^3, it was around 46% higher than under continuous illumination.

In the first 100 hours, the cell growth under continuous illumination reached a maximum at 1.7 10^9 cells/dm^3 while under light-dark cycle illumination was lower, around 8.0 10^4 cells/dm^3. But with longer cultivation, reaching 200 hours, cell growth under photoperiodicity of 8 and 9 hours per day showed that cells density was higher than the maximum cell density under continuous illumination. The cell density under photoperiodicity of 8 and 9 hours per day was approximately 40% higher than under continuous illumination. This is due to the availability of adequate light energy during cell metabolism process in the early stage of cultivation under continuous illumination, so that *Chlorella vulgaris* Buitenzorg is able to grow rapidly. But in the middle of cultivation stage, the growth rate was lower than the previous stage since *Chlorella vulgaris*

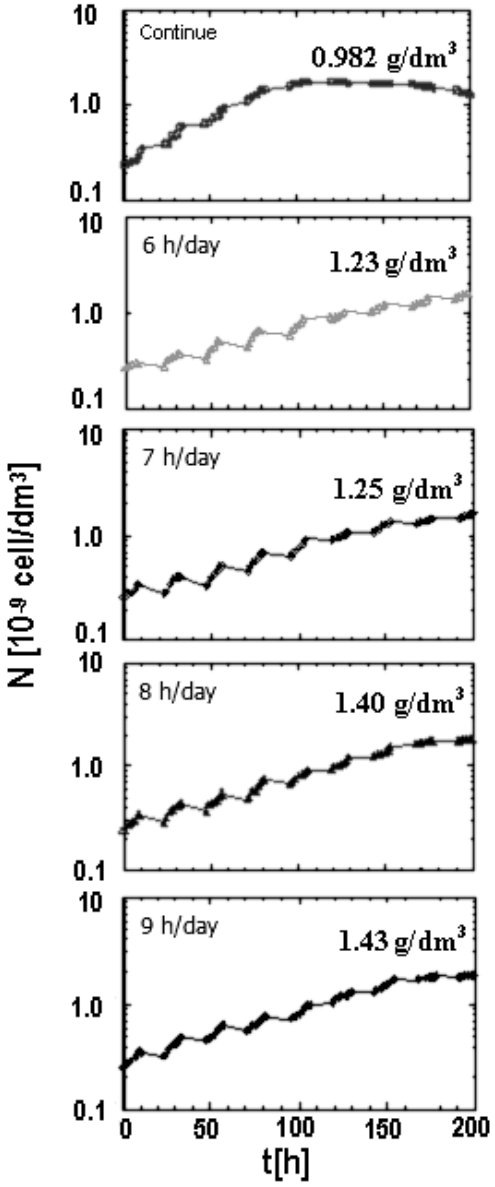

Figure 4. The Growth curve of *Chlorella vulgaris* Buitenzorg

Buitenzorg reached the stationary phase. At the end of the process, number of cell started declining. This is appropriate to the result of previous research [12,13]. Values of *Chlorella vulgaris* Buitenzorg growth rate μ that is defined as an incidentally growth rate is shown in Figure 5. This value was calculated by the following equation.

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \quad \text{or} \quad \mu = \frac{1}{N} \cdot \frac{dN}{dt} \qquad (1)$$

Under continuous illumination, in the beginning of cultivation stage, the growth rate (μ) was high, but μ declined at the end of exponential stage. At photoperiodesity, the growth rate at the first day was lower and declines to zero in the dark period. At the next light period, the growth rate tend to increase and also declines to zero growth in the dark period. An increasing growth rate was also happened in the next days light period. It caused by a formation of cellular *chlorophyl a* during dark period which will increase the capability of light energy absorption in the next light period; it is also caused by capability of cellular DNA formation, RNA formation and cell division [4-6, 14]

High cell metabolism during cell growth activity could increase the pH of the medium. The cellular metabolism which is carried out inside the micro algae cell produces an alkaline condition, base on the intercellular metabolism reaction.

$$H_2O + HCO_3^- \rightarrow \tfrac{1}{6} C_6H_{12}O_6 + O_2 + OH^- \qquad (2)$$

CO_2 from bubbling gas enter the medium in the form of bicarbonate substances. This bicarbonate substance [HCO_3^-] is absorbed by the cells of *Chlorella vulgaris* Buitenzorg. Subsequently, the cell metabolism process will produce organic substances such as glucose and OH^- ion as written in the equation 2 above. The amount of calculated carbonate ion reflects the amount of carbonate ion attained in *Benneck* medium during cell culture cultivation. The dissolved carbonate ions is consumed by micro algae to maintain cellular metabolism or CO_2 pools in cell vacuole, inside micro algae cell structure [15].

From the data of pH of culture medium during cell cultivation, [HCO_3^-] can be calculated using the Handerson-Hasselbach equation, that is shown at equation 3. Here, A_K, B_K, C_K are the parameter of CO_2 equilibrium constant, while A_H, B_H, C_H are the Henry constant. $H_{CO2,o}$ is ambient Henry constant, Y_{CO2} is ambient CO_2 concentration in air, $K_{CO2,o}$ is ambient equilibrium constant.

$$[HCO_3] = \left[\frac{\exp[A_k(1-\frac{T_o}{T}) + B_k \ln(\frac{T}{T_o}) + C_k(\frac{T}{T_o}-1)]}{\exp[A_H(1-\frac{T_o}{T}) + B_H \ln(\frac{T}{T_o}) + C_H(\frac{T}{T_o}-1)]} \right] \cdot \left(\frac{K_{CO_2,o}}{H_{CO_2,o}} \right) \cdot \left(\frac{Y_{CO_2} \cdot P_T}{10^{-pH}} \right) \qquad (3)$$

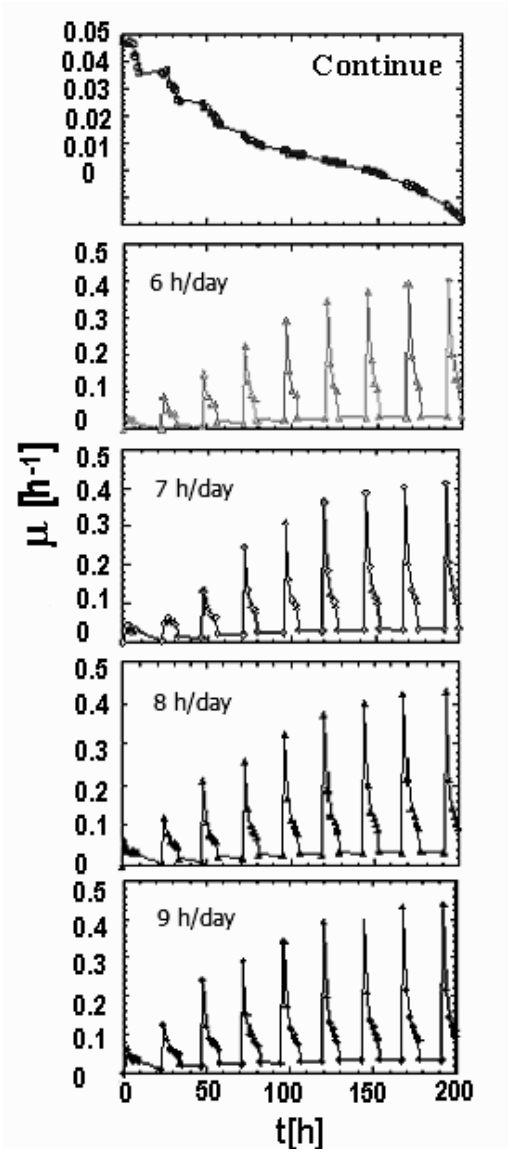

Figure 5. Growth rate of *Chlorella vulgaris Buitenzorg*

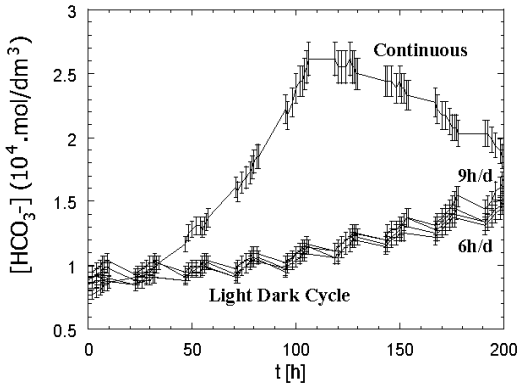

Figure 6. [HCO_3^-] a predicted substrate content in medium

Figure 6 shows [HCO_3^-] of culture media during cell cultivation at continuous illumination and photoperiodesity. It is obviously seen that the decrease of [HCO_3^-] in the medium, is in accordance with the reverse reaction of equation 2, (cellular maintenance metabolism) and the released CO_2 from CO_2 pools in cell vacuole, inside micro algae cell structure. This result is also in compliance with the result of previous research, showing that [HCO_3^-] will continuously increase during cultivation period.

Alteration of *I* is caused by an increase of population density of *Chlorella vulgaris* Buitenzorg so the demand for light energy to maintain metabolism process for cellular growth will increase.

Table 1 shows the effect of alteration of *I* on biomass energy consumption and energy efficiency for biomass production of *Chlorella vulgaris* Buitenzorg, during culture cultivation under continuous illumination and under photoperiodicity.

Alteration mode from continuous illumination to light-dark cycle illumination, showed that energy efficiency and energy consumption for biomass formation increased around three times. Alteration of illumination mode from continuous illumination to photoperiodicity is also related to shadow effect of the *Chlorella vulgaris* Buitenzorg itself that was happened during the cultivation. The shadow effect will change the amount of *chlorophyl a* content in *Chlorella vulgaris* Buitenzorg, as explained in a research carried out by Paul G. Falkowaski and Thomas G. Owen [9]. Their experiment showed that sunshine energy consumption tend to increase the cellular metabolism; that is why the biomass energy consumption under photoperiodesity were higher than under continuous illumination.

Carbon Transfer Rate or CTR ($g.dm^{-3}.h^{-1}$) is the amount of CO_2 gas transferred per volume of medium from bubbling gas to liquid medium. A part of transferred

Table 1. The biomass energy consumption and energy efficiency for biomass production of *Chlorella vulgaris* Buitenzorg

	Energy efficiency	Biomass energy consumption
6 h/day	18.9 %	498 J/g
7 h/day	17.2 %	577 J/g
8 h/day	15.2 %	683 J/g
9 h/day	13.8 %	766 J/g
Kontinu	5.62 %	2070 J/g

CO_2 is utilized for cell metabolism during its cultivation period. CTR assessment is determined by calculating the change of CO_2 concentration that carried out by measuring the CO_2 concentration before entering the medium and after passing the reactor, using Gas Chromatography Analyzer. With calibration constant α of 53.3 $g.dm^{-3}.h^{-1}$, we can calculate the value of CTR [16]. Culture medium CTR during cell cultivation in both of continuous illumination and photoperiodesity are shown in the Figure 7. q_{CO2} is defined as the amount of CO_2 gas transferred due to biological life activity in a certain volume of medium and in a certain period of time. Value of q_{CO2} could be calculated by dividing CTR by biomass dry weight (X).

Figure 7 shows that the mean value of q_{CO2} under continuous illumination was 1 x 10^4 h^{-1}, and under light-dark cycle illumination were around 1.25 – 1.8 x 10^4 h^{-1}.

This shows that the q_{CO2} of photoperiodesity is better than the continuous illumination. For both illumination, CTR were declining. These are related to cell activity, where the cell activity is high at the beginning and low at the end of the process, so that the medium is saturated with CO_2 and there will be no more CO_2 entering the medium. This is in compliance with the previous research [17], which stated that the change of CO_2 inside the cell transformed into organic substances caused a decrease of the amount of CO_2 inside the medium. In other words increasing growth rate during cell cultivation or biomass production under photoperiodesity, directly increasing the culture-q_{CO2}. This means that the average q_{CO2} of the culture under photoperiodicity is higher than under continuous illumination.

Growth model of *Chlorella vulgaris* Buitenzorg is created as a mean for the predict on the simple empirical correlation between growth of *Chlorella vulgaris* Buitenzorg with the availability of substrate in the medium. The result of the prediction is shown in Figure 8 and 9.

The model prediction is chosen among several models based on the curve regression of the experimental results.

Monod Model

$$\mu = \mu_{\max} \cdot \frac{[HCO_3^-]}{K_s + [HCO_3^-]} - K_e \qquad (4)$$

Ierusalimsky Model

$$\mu = \mu_{\max} \cdot \frac{[HCO_3^-]}{K_s + [HCO_3^-]} \cdot \frac{1}{\left(1 + \frac{[HCO_3^-]}{K_I}\right)} - K_e \qquad (5)$$

Haldane Model

$$\mu = \mu_{\max} \cdot \frac{[HCO_3^-]}{K_s + [HCO_3^-]\left(1 + \frac{[HCO_3^-]}{K_I}\right)} - K_e \qquad (6)$$

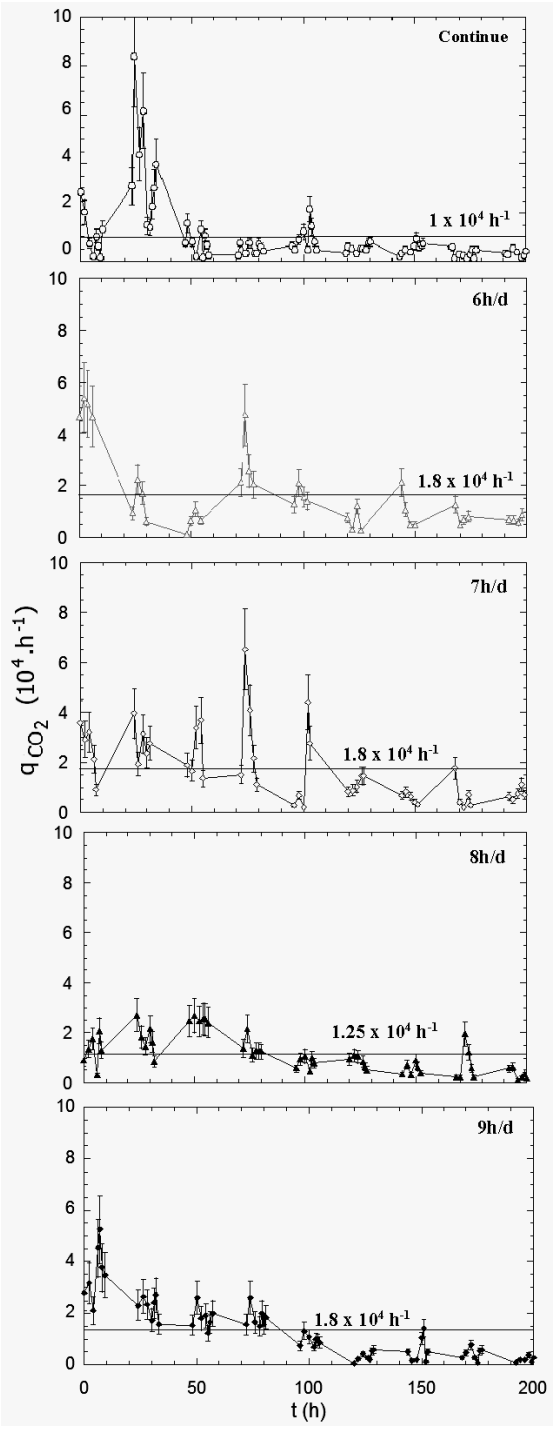

Figure 7. The Changes of q_{CO2} at both of continuous illumination and photoperiodesity

μ is defined as growth rate (h^{-1}), μ_{max} is maximum growth rate (h^{-1}), K_s is substrate constant, K_I is inhibition constant and K_e is endogoneus constant [18]. Under continous illumination (Figure 8), the best prediction model followed Haldane model, with the error deviation about 2.8%.

Under photoperiodicity (Figure 9), the most appropriate model prediction also follows Haldane model. Evaluation result of the appropriate model was concluded by combining the approached prediction of both of dark and light period condition. The error deviation with this equation is around 2.5-3.2%, and the K_e number for the light condition was near to zero and for dark condition were around 0.27-1.35.

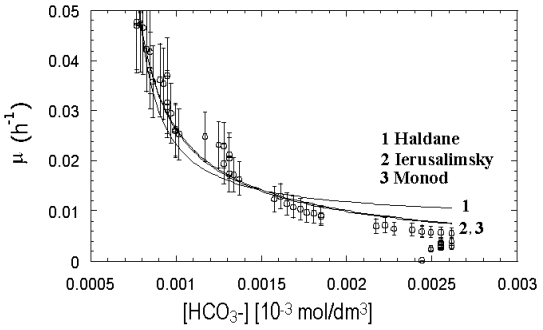

Figure 8. Empirical prediction of kinetic model of micro algae growth under continuous illumination

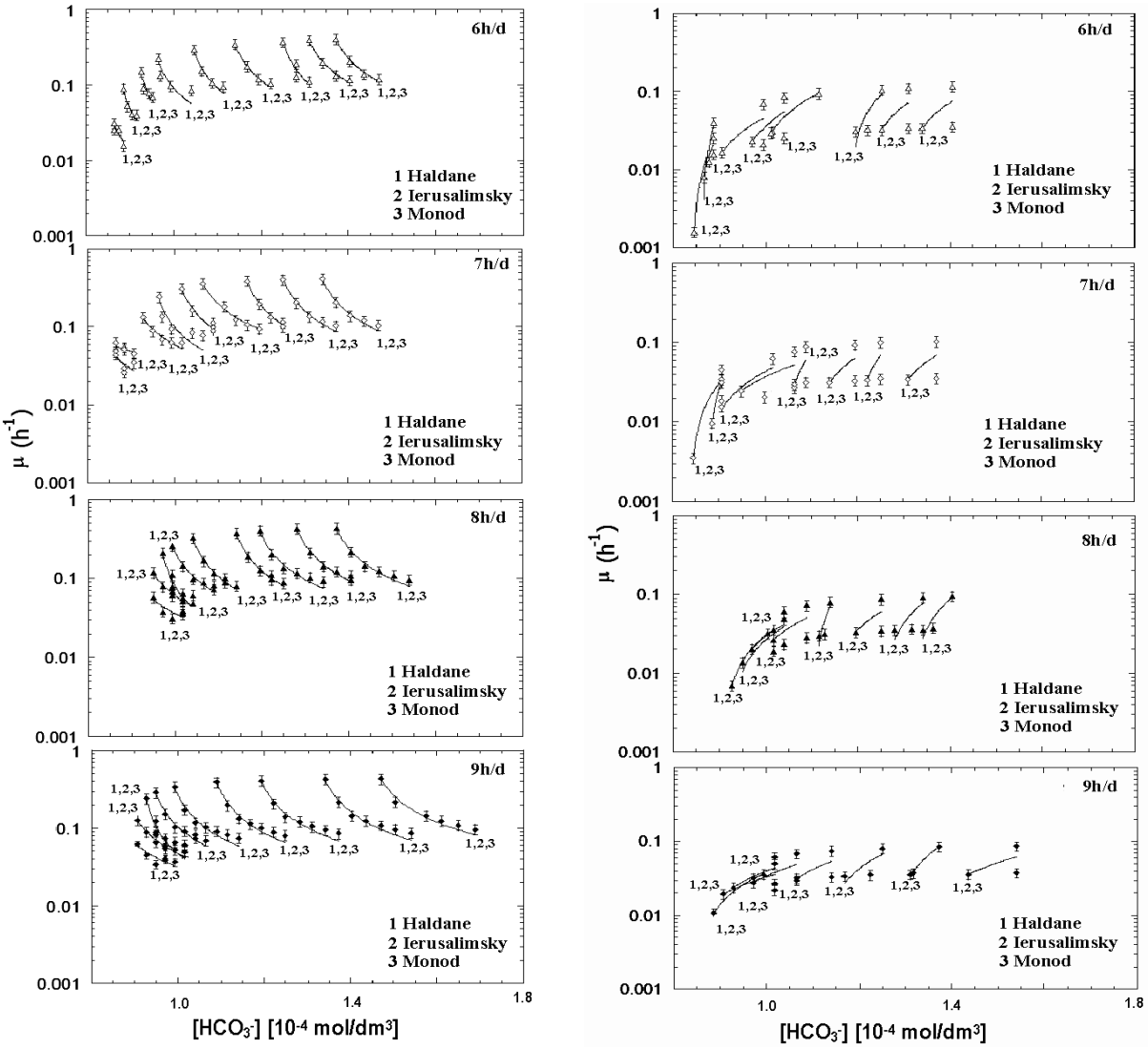

Figure 9. Empirical prediction of kinetic model of micro algae growth on left. light period and right dark period

The result of this prediction result was similar to the previous result of the research on *Anabaena cylindrica* [16,18,19], which concluded that kinetics model of small scale photosynthetically microalgae followed competitive inhibition kinetics model.

4. Conclusion

Under photoperiodicity of 8 and 9 hours per day, the culture cell density of N were approximately 40% higher than that under continuous illumination. Final biomass density of *Chlorella vulgaris* Buitenzorg at 9 h/d illumination was 1.43 g/dm^3, around 46% higher than that under continuous illumination.

q_{CO2} under photoperiodicity was higher than under q_{CO2} continuous illumination. Under photoperiodicity, q_{CO2} increased around 50-80% compared to q_{CO2} under continuous illumination.

This experiment showed that photoperiodicity effects the growth of *Chlorella vulgaris* Buitenzorg, where the growth rate (μ) under photoperiodicity is higher than under continuous ilumination, although the growth period is two times longer.

Photoperiodicity also save consumption of light energy and increasing light energy efficiency. Other result showed that Haldane model was the fitted empirical prediction of kinetic model of micro algae growth under continuous illumination. The same empirical prediction kinetics model of micro algae growth under light/dark cycle of illumination hours per day (τ_i) lso fitted to Haldane model.

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