

Enhancement Of Cyanobacteria Growth In Serial Configuration Photobioreactor By Photon Flux Density Alteration

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Abstrak

Masalah pemanasan global yang disebabkan oleh peningkatan gas CO₂ di udara mendorong upaya aktif untuk mengatasinya. Salah satu alternatif yang efektif yaitu dengan menggunakan mikroalga *Spirulina platensis* disebabkan selain kemampuannya dalam memfiksasi CO₂, produksi biomassa yang dihasilkan juga sangat bermanfaat bagi kehidupan. *Spirulina* mempunyai kandungan protein yang tinggi serta mengandung zat aktif untuk mengatasi berbagai macam penyakit seperti kanker serta mengurangi kadar kolesterol dalam darah. Adanya potensi-potensi tersebut membuat riset difokuskan untuk peningkatan produksi biomassa *Spirulina platensis*. Metode yang digunakan adalah perlakuan alterasi pencahayaan dimana intensitas cahaya yang diberikan akan ditingkatkan sesuai dengan peningkatan biomasanya. Kultivasi akan berlangsung dalam fotobioreaktor yang disusun secara seri dengan volume masing-masing sebesar 500 mL dengan menggunakan medium Conwy sebagai sumber nutrisi dan pada temperatur 29 °C serta tekanan operasi 1 atm. Sebagai sumber karbon digunakan gas CO₂ dengan konsentrasi masukan sebesar 3% dengan kecepatan superfisial sebesar 1,2 m/h. Sumber cahaya yang digunakan berasal dari lampu Phillips Halogen 20W/12V/50Hz. Sebagai eksperimen banding dilakukan kultivasi *Spirulina platensis* dengan menggunakan metode pencahayaan kontinu (Intensitas yang diberikan nilainya tetap). Perbandingan hasil produksi akhir metode alterasi ini rata-rata lebih tinggi 55,1 % dibandingkan dengan metode pencahayaan sinambung. Selain itu energi yang diperlukan pada pencahayaan alterasi juga lebih sedikit bila dibandingkan pencahayaan kontinu yakni hanya sebesar 21,6% dari energi yang dibutuhkan pada pencahayaan sinambung.

Kata kunci: *Spirulina platensis*, model kinetika Substrat dan Jerusalemky.

Abstract

There are many researches to solve the effects of global warming caused by great amount of CO₂ in the air. One of the effective alternatives to reduce this gas in atmosphere is by using micro alga *Spirulina platensis* due to its ability of CO₂ fixation and the very useful biomass that it produced. *Spirulina platensis* contains high protein and can cure diseases such as cancer and cholesterol reduction. In considering of these benefits, this research focused on increasing the biomass production of *Spirulina platensis* by alteration of light illumination during microbial growth. The cultivation holds in a series of photobioreactors at 29°C and 1 atm where each of photo-bioreactor has volume of 500 mL, using Conwy medium as nutrition. 3% CO₂ is the carbon source for the cultivation with superficial velocity 1.2 m/h. Phillips Halogen lamp 20W/12V/50Hz is the source for illumination. The cultivation using constant intensity of light illumination was also be done as a control. Cultivation of *Spirulina platensis* with alteration illumination method successfully increased the biomass production 55.1 % higher than constant intensity of light illumination. The energy of producing biomass in alteration of light illumination method lower than continuous intensity illumination which was only 21.6 % than constant intensity of light illumination. Kinetic studies of this microbial growth at alteration of light illumination also concluded that specific growth rate and bicarbonate concentration as essential compound followed Jerusalemky kinetic model equation.

Keywords: *Spirulina platensis*, substrate kinetics model and Jerusalemky,

1. Introduction

As the science and technology updated, there are also negative side following. One of it is the global warming. Global warming exists because of the raise of CO₂ in the atmosphere because of the activity and consumption of fossil fuels [1]. Many of researches have been done to fix this problem, and one of the effective alternatives is using micro alga, which have ability to reduce CO₂ by photosynthesis reaction. Micro alga are also one of healthy food.

Micro alga is rich in nutrition, contains many vitamin, β -caroten, and others. These benefits encourage researches on optimizing the Micro alga growth on such topics,

1. The effect of photoperiodicity in the micro alga growth as reported by Y. K. Lee and S. J. Pit [2]
2. Alteration of light illumination in micro alga growth to increase the biomass production of *Anabaena cylindrica* as reported by A. Wijanarko and K. Ohtaguchi [3].
3. The biomass production of *Chlorella vulgaris* Buitenzorg with the optimization periodic illumination in series reactor reported by Muryanto [4]
4. The biomass production of *Chlorella vulgaris* Buitenzorg with optimization of alteration of light illumination by A. Y. Sendjaja [5].

One of other promising Micro alga is *Spirulina sp.*, the single cell-blue-green micro alga (*Cyanobacteria/Cyanophyta*). The high capacity of cell production and the ability to construct a colony make *Spirulina sp.* easy to produce in big scale. *Spirulina sp.* is classified as a blue-green Micro alga because it has both of chlorophyll and phycocyanin pigment in the cell structure [6].

Spirulina sp. has very high protein content which is around 60-70% of its dry weight [7]. Beside as a food resource, *Spirulina sp.* also can be used as medicine and cosmetic. *Spirulina sp.* has a rapid cell

reproduction in alkali environment, easy to growth in monoculture, every cell can be utilized, and nontoxic. *Spirulina sp.* also has a phycobiline pigment which could be used to increase the antibody [8].

Considering all those reasons we will cultivated *Spirulina sp.* in Laboratory scale using the photobioreactor to growth *Spirulina sp.* Photosynthesis is part of *Spirulina sp.*'s life cycle. Thus light is the important factor in its growth. This research is directed to maximize the growth of *Spirulina sp.* with variation of light intensity that given to the culture during the cultivation. Alteration of light is a method where the light intensity that given to the culture equivalent with the biomass concentration in the reactor. This research used alteration of light intensity because the light required by culture is increasing along with the increasing of cell number. This research also used series reactor due to the previous research that proves the ability of series reactor on increasing the number of biomass of *Spirulina sp.*

The objective of this research was to increase the biomass production of *Spirulina sp.* This research expected to be used as a start for developing the Micro alga *Spirulina sp.* on larger scale in the future.

2. Materials and Methods

Spirulina platensis that used in this research was supplied from Balai Perikanan dan Kelautan Jakarta. The culture was grown in three series photobioreactors, 500 mL each (Figure 1). Every reactor contained Conwy medium which also supplied from Balai Perikanan dan Kelautan Jakarta. This culture was aerated by 3% CO₂ enriched air which was illuminated at alteration of light intensity start at 5.77 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. The illumination would be increased as the number of increased cell by changing the distance between reactors and Phillips Halogen Lamps. Growth temperature was 300 K and superficial gas velocity U_G was 1.2 m/h. Cultivation at constant light illumination of

5.79 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ was also performed as control experiment.

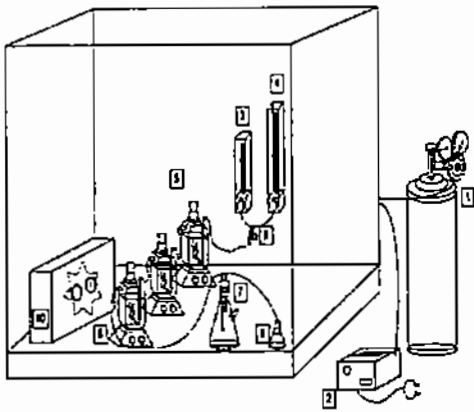


Figure 1.

Experimental apparatus, CO₂ bomb (1), Portable Air Compressor (2), CO₂ Flowmeter (3), Air Flowmeter (4), Photobioreactor (5), Stirrer Plate (6), Outlet CO₂ Indicator (7), Discharge CO₂ Erlenmeyer (8), Inlet CO₂ Indicator (9), Illumination Source (10)

Measuring optical density at 680 nm of cell suspensions was performed for biomass production. The calibration curve that showed relation between OD and dry was made in order to measure the dry weight easily by only measuring the OD in Spectrophotometer. The number of cell started in $4 \cdot 10^6 \text{ cell}/\text{cm}^3$. The illumination measured by Luxmeter and pH was also measured as growth parameter.

3. Result and Discussion

The cultivation of *Spirulina platensis* with alteration illumination was performed in $4 \cdot 10^6 \text{ cell}/\text{cm}^3$ or $0.106 \text{ g}/\text{dm}^3$ at the beginning and the illumination intensity started at $5.77 \mu\text{mol}/\text{m}^2\cdot\text{s}$. The illumination was gradually increased as the number of cell upgraded by looking the $I_{\mu\text{maks,opt}}$ graph (Figure 2) that had already made before [9].

The intensity given in the research can be seen in the graph below (Figure 3). The alteration of light intensity started from $5.7 \mu\text{mol}/(\text{m}^2\cdot\text{s})$ until the maximum intensity for *Spirulina sp.* ($21.45 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$). But for cultivation at constant light illumination, the intensity did not change.

3.1. The effect of alteration illumination on *Spirulina platensis* growth

Alteration light illumination method proved that it could increase the biomass production of *Spirulina platensis*. The number of dry weight at the end of cultivation is illustrated in Table 1.

The differences of biomass production between alteration of light illumination and the illumination at constant intensity are depicted clearly in the graph below (Figure 4).

Based on these graph and table, the alteration of light illumination could increase biomass production of *Spirulina platensis* up to 55.14 %. This was because as the number increase, the cells needed more light to maintain their growth. The alteration of light illumination method, made the cell growth in maximum point. On the other hand, the increasing of biomass production at constant intensity of light illumination was not followed by the increasing of light intensity given to the culture. This make the diversity of light was not distribute fairly because of the dense population of the culture. This phenomenon is usually called as self shading effect [10]. It makes the cells cultured in constant intensity illumination method growth in less optimum growth point so the biomass production is less than cells cultured on alteration light illumination method.

This was also supported by *Spirulina platensis* specific growth rate in alteration of light illumination which was higher than in constant intensity cultivation (Figure 5). The *Spirulina's* specific growth rate in the beginning of cultivation (μ_{max}) of this alteration was 0.139 h^{-1} , higher than in constant intensity (only 0.062 h^{-1}).

This means that alteration of light illumination can increase the specific growth rate of the cell. It was because in the alteration of light illumination, the growth of cell kept constant in higher point even when the intensity of illumination increased. This was where the cell population met the stationary phase (phase

when there is an equal number of the new cells and dead cells). The growth of the cell can be measured with this equation below

The growth of cell in alteration and constant intensity light illumination can be seen in the graph below.

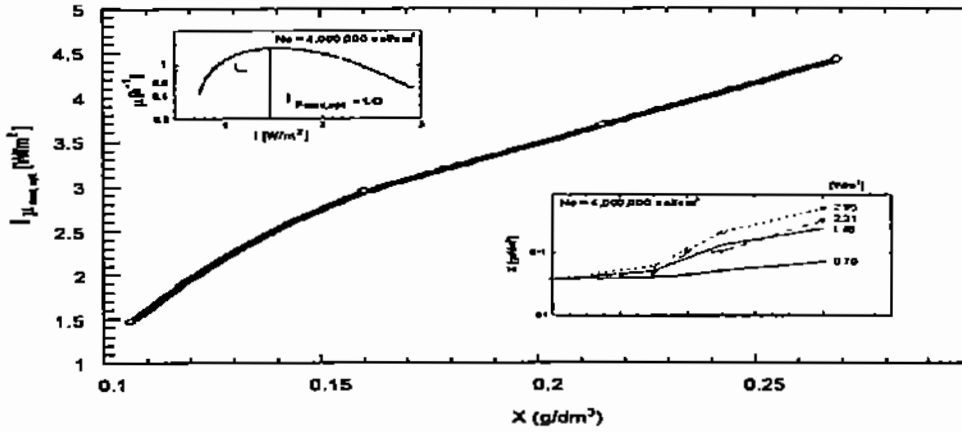


Figure 2.
 $I_{\mu_{max,opt}}$ values (X) [9]

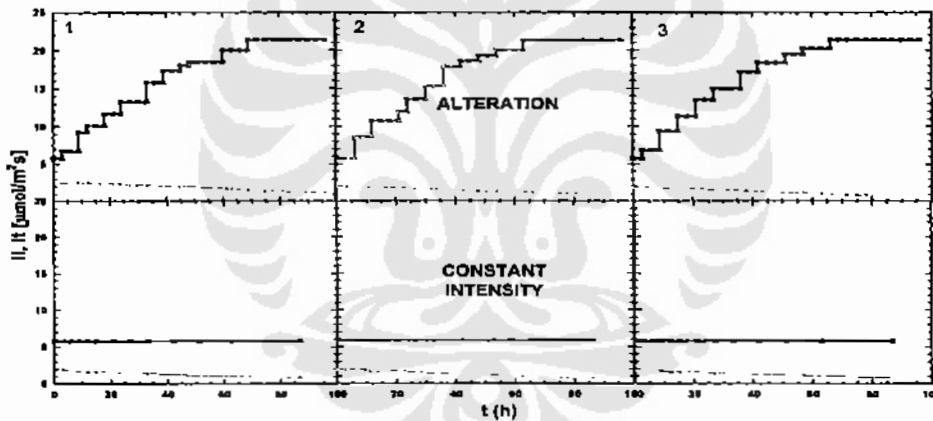


Figure 3.
Illustration of Alteration and Constant of Light Illumination, Illumination Measured Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

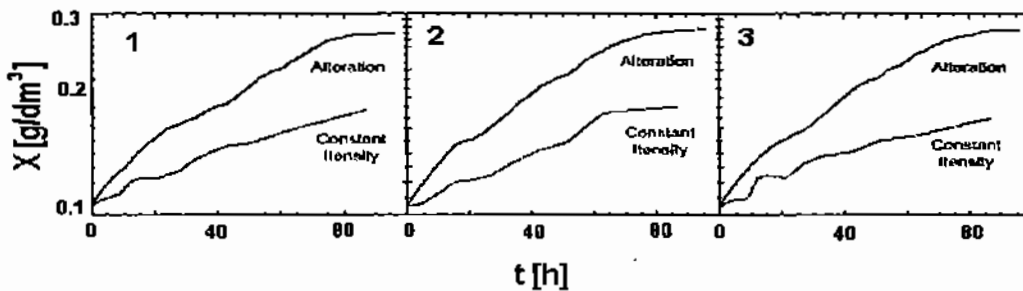


Figure 4.
Graph of Cell's Dry Weight (X) vs Time (t); cell's Dry Weight Measured Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

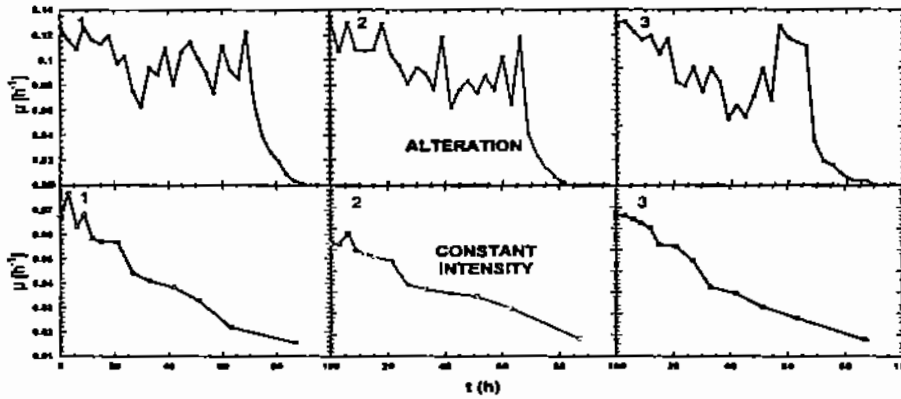


Figure 5.

Specific Growth Rate in Alteration and Constant of Light Illumination, Specific Growth Calculated Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

Tabel 1
Final Cell Dry Weight

Illumination	Reactor	X final (g/dm ³)
Alteration	1	0.270
	2	0.276
	3	0.274
Constant (5.77 μmol/m ² .s)	1	0.178
	2	0.181
	3	0.170

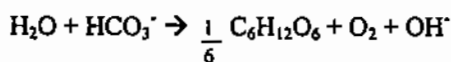
$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \quad (1)$$

$$E_x = \frac{\int_0^t I_t dt}{\Delta X \cdot s} \quad (2)$$

The number of I_t showed that it decreased until the end of cultivation. This means that the consumption of energy was increasing as the concentration of the cell increase. The calculation of E_x can be seen in Table 2.

3.2. The Effect of Alteration of Light Illumination in Medium pH

The metabolism of cells on its life cycle increased the pH of medium and it keeps happened until the end of cultivation as shown on Figure 6 below. It happens because of the activity of the cell was high until the end of the cultivation and the cell grew in the highest point. The cell metabolism resulted on releasing of OH^- . As the metabolism was kept on maximum level, the OH^- released also high. This was why the medium pH increased until the cultivation reached the end. The reaction can be seen below.



3.3. The Energy of Spirulina Platensis Biomass Production (E_x)

To calculate E_x we used I_t data that showed the intensity that transmitted by cell and can be calculated with this equation.

Tabel 2
Nilai E_x yang Diperoleh pada Penelitian

Illumination	Reactor	E_x (J/g)
ALTERATION	1	269.99
	2	257.92
	3	237.46
CONSTANT	1	939.15
	2	1739.45
	3	1292.31

Based on the calculation, the energy used to biomass production (E_x) in alteration of light illumination is 21.6 % smaller compare to the energy required in constant intensity. The biomass production in alteration of light illumination is higher than constant intensity, so it lessening the intensity transmitted from the reactor (I_t). This made the integral of I_t smaller. Both of those things made the energy per unit biomass lesser and this was suitable with the equation above.

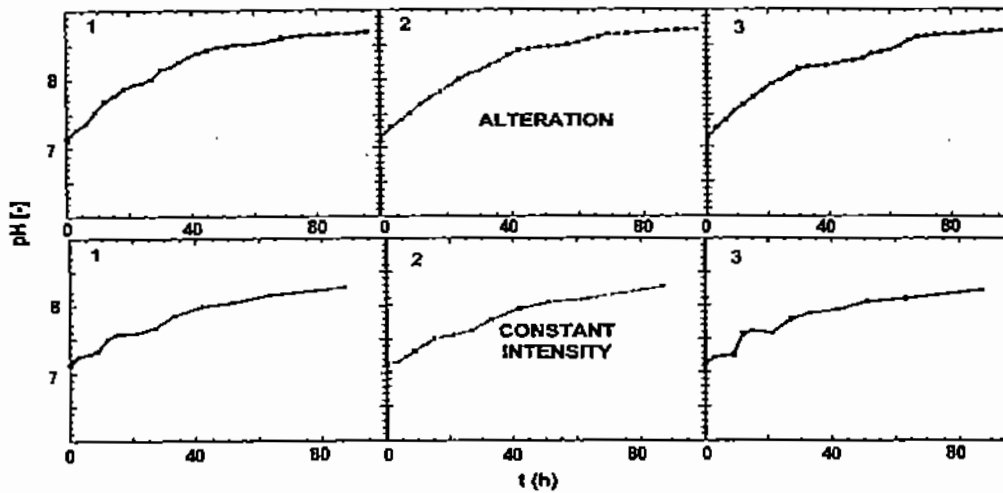


Figure 6.

The Graph of pH vs Time, pH Measured Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

3.4. The Effect of Alteration of light Illumination to The Bicarbonate $[HCO_3^-]$ Concentration in The Medium.

The bicarbonate concentration was calculated to find out the number of bicarbonate concentration in the medium that can be consumed with *Spirulina platensis* for its metabolism. Figure 7 showed that bicarbonate concentrations in the medium for both methods was nearly the same (both have an increasing bicarbonate concentration). But when the cultivation reached the end, the concentration increased insignificantly and at the end it becomes constant. The number of bicarbonate concentration that soluble in the medium in the alteration of light illumination method was 177.8% higher than in constant intensity. This is because in the alteration of light illumination method, the substrates absorb more bicarbonate than in constant intensity. Thus the bicarbonate concentration desired was also higher than constant intensity and it was caused by the bicarbonate soluble in the medium of alteration was more than constant intensity.

3.5. Substrate absorption kinetics model

There were three equations of kinetic models that used to investigate the

microbial growth model in this research as a substrate inhibition model [11]. Such as:

Monod Equation

$$\mu = \mu_{max} \cdot \frac{[HCO_3^-]}{K_S + [HCO_3^-]} \quad (3)$$

Monod equation is the equation to find the rate of cell reproduction with assumption that cell growth is a function of substrate concentration without considering other factors [12]

Ierusalemky Equation

$$\mu = \mu_{max} \cdot \frac{[HCO_3^-]}{K_S + [HCO_3^-] \left(1 + \frac{[HCO_3^-]}{K_I}\right)} \quad (4)$$

This equation describes a specific growth rate that is influenced by non-direct competitively product inhibition factor.

Haldane Equation

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

This equation describes a specific growth rate model that is influenced by competitive reaction product.

From the experimental results calculated using those equations, plots of cell population kinetics illustrating relationship

between the specific growth rate (μ) and bicarbonate ion concentration as an essential compounds [S] were made and the results of the calculations, are represented as shown in Figure 8, Table 3 and Table 4.

The *Spirulina platensis* growth model at the alteration of light illumination and the

constant light illumination is following the Ierusalemky equation showed in Figure 6. Examination of the curves plot refers to the cells population kinetic equations presented in previous explanation and to substrate inhibition equation as indicated in Table 3 and Table 4.

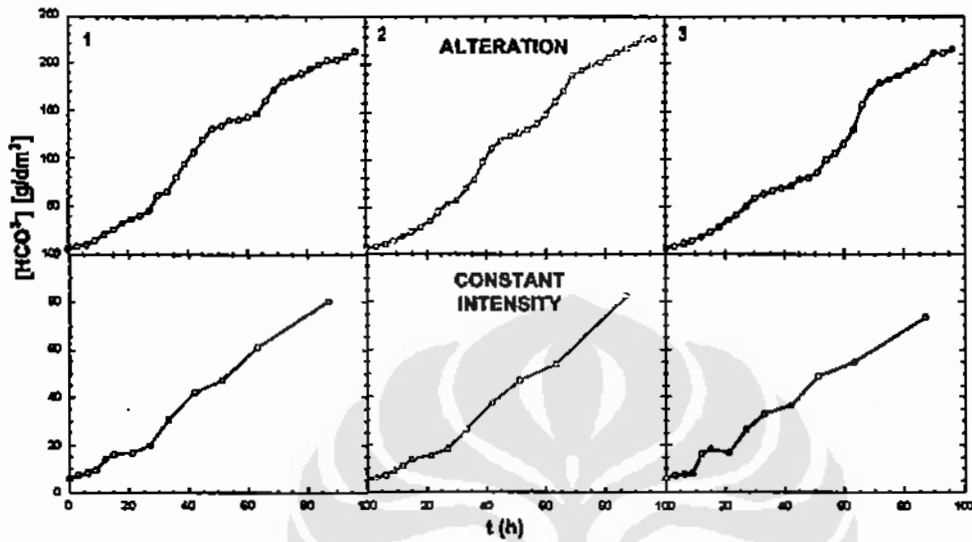


Figure 7. Bicarbonate Concentration $[HCO_3^-]$ vs Time; HCO_3^- Concentration Measured Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

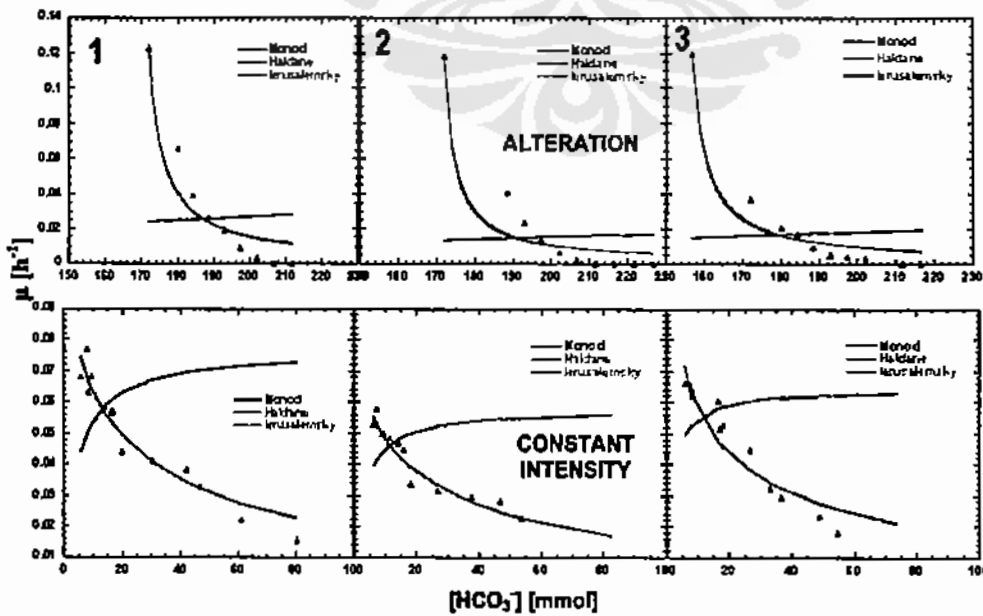


Figure 8. Kinetic Model of The Culture, Kinetic Model Result of First Reactor in a Series of Photobioreactors (1); Second Reactor (2); and Third Reactor (3).

Table 3.
Results of The Calculation of Kinetic Model in Alteration of Light Illumination

Equation	Reactor	μ_{max}	K_S	K_I	R
Haldane	1	0.122	-3945.3	7.499	0.959
	2	0.118	-5845.3	5.058	0.958
	3	0.119	-3648.7	6.741	0.956
Ierusalemky	1	0.122	-168.05	4.04	0.962
	2	0.118	-169.19	2.808	0.961
	3	0.119	3.78	3.782	0.96
Monod	1	0.122	706.69	-	~
	2	0.118	1347.9	-	~
	3	0.119	1101.6	-	~

Tabel 4.
Results of The Calculation of Kinetic Model in Constant Light Illumination

Equation	Reactor	μ_{max}	K_S	K_I	R
Haldane	1	0,077	-0,817	33,595	0.964
	2	0,058	-0,852	5,058	0.961
	3	0,064	-1,508	36,185	0.937
Ierusalemky	1	0,077	-0,719	0,328	0.966
	2	0,058	-0,0746	33,210	0.962
	3	0,0644	-0,1333	34,593	0.940
Monod	1	0,077	4,356	-	~
	2	0,058	2,909	-	~
	3	0,064	1,754	-	~

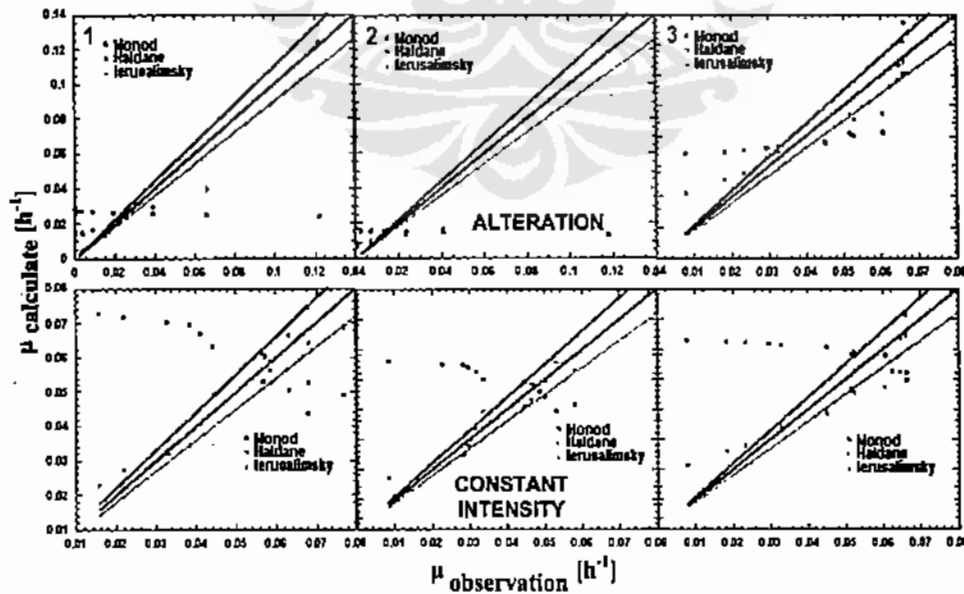


Figure 9.
Deviation of Kinetic's Model, Deviation Result of First Reactor in a Series of Photobioreactors (1);
Second Reactor (2); and Third Reactor (3).

So if we refer to error results, shown in Figure 9, we can see that Ierusalemky equation gives the number of errors mostly lesser than 10%. Considering this we can conclude that the most suitable kinetic model in this research is Ierusalemky equation. This result was quite similar to spirulina's growth characteristic trend which was tend a non direct competitively inhibition reaction phenomenon during it culture cultivation.

4. Conclusions

Alteration of light illumination can increase the biomass production of *Spirulina platensis* (X) up to 55.57% higher than constant light illumination. The medium pH in alteration of light illumination was also quite high until the end of the cultivation because of the activity of the cell. The bicarbonate concentration in the medium was also 26.9% higher than constant light of illumination. The energy that used to biomass production of *Spirulina platensis* (E_x) in alteration of light illumination was only 21.6 %, which lesser than energy needed in constant light of illumination. The experimental results of the microbial growth at alteration of light illumination also confirmed that relationship between the specific growth rate (μ) and bicarbonate ion concentration as an essential compounds concentration as *Spirulina* growth population followed a substrate inhibition model kinetic equation that was proposed by Ierusalemky.

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