

## Research Article

# The Effect of Liquid Organic Fertilizer “Bio Ferti” Application on the Growth Rate of *Spirulina platensis* by Using Haldane Model

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### ABSTRACT

This experimental research was performed to observe the influence of an agricultural liquid organic fertilizer called Bio Ferti on the growth and biomass of *Spirulina platensis*, aiming at replacing inorganic fertilizer with the liquid organic one. The cultivation of the microalgae was conducted over seven days at Nogotirto Algae Park. The liquid organic fertilizer, namely Bio Ferti, was obtained from the Faculty of Biology, Universitas Gadjah Mada, and prepared to have doses of 2, 4, 6, 8, and 10 mL. For comparison, an inorganic fertilizer with the same doses was also prepared. The variables to be observed were cell density, dry cell weight, and growth kinetics. The culture medium conditions observed were temperature, pH, and salinity (the optimum salinity was 20 ppt). The growth kinetic analysis was performed mathematically using numerical simulations using the Contois and the Haldane models. This research's results showed that Bio Ferti affected the growth rate of *Spirulina platensis*. With a dose of 2 mL, it became the optimum medium which produced the highest density and dry weight of  $1.78 \times 10^6$  cells/mL and 160 mg/mL, respectively. Meanwhile, the inorganic fertilizer with a dose of 10 mL produced the highest density and dry weight of  $2.13 \times 10^5$  and 80 mg/mL, respectively. The temperature ranged from 28 to 31°C, while the pH ranged from 8.01 to 9.02 for each medium. The suitable model to describe the growth kinetics of *Spirulina platensis* was the Haldane model.

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### NOMENCLATURE

$\mu_m$	[day <sup>-1</sup> ]	maximum specific growth rate
$K_s$	[mg/L]	half saturation constant
$Y_{x/s}$	[mg cell]	yield of cell formation
$k_d$	[day <sup>-1</sup> ]	death rate constant
$K_I$	[mg/L]	inhibition constant

## INTRODUCTION

Microalgae are biomass potential to develop. As fast-growing unicellular or simple multicellular microorganisms, they provide several advantages, including higher photosynthetic efficiency, higher growth rates, and higher biomass production, compared to other energy crops (Li et al. 2008; Gouveia et al. 2009). *Spirulina platensis*, known as the Blue-Green alga, is multicellular, filamentous, and unbranched blue-green microalgae, which is naturally grown in alkaline waters in warm regions (Bandara & Arunakumara 2020). It can colonize in a wide range of environments that are not suitable for many other living organisms (Madkour et al. 2012). Although possessing a simple prokaryotic cell structure, it has no plant cell wall but the photosynthetic ability and glycogen-containing cellular membrane, making it like the bacterial, plant, and animal kingdoms, respectively (Usharani et al. 2012).

*Spirulina platensis* can produce valuable metabolites, such as proteins, fatty acids, and pigments for feed additives and nutritional purposes (Guerin et al. 2003; Hu. 2004). Among filamentous Cyanobacteria, *S. platensis* is preferred for biomass production mainly due to its relatively high cell growth rate and easy biomass recovery (Pioreck et al. 1984). In addition, its ability to grow in highly alkaline media reduces the risk of being contaminated by other microorganisms (Walach et al. 1987).

*S. platensis* has high adaptability, which makes it easier to be cultivated in various nutrient conditions. Generally, Zarrouk and Walne media are used to cultivate it on a large scale. However, both media are relatively complex, expensive, and limited, bringing the production cost to rise. For this reason, some researchers have begun to search for other media to minimize the production cost and increase revenue.

There are very few studies on the use of agricultural fertilizers for cultivating macro and microalgae (Ilknur 2011). In recent years, consumers preferred to use organic media such as Zarrouk, Walne, and many more, as generally preferred by *S. platensis* cultivators. Some investigators reported the replacement of several elements contained in the Walne and Zarrouk media like organic matters (Andrade & Costa 2009) and wastewater (Mezzomo et al. 2010) to be used as sources of nutrients for *S. platensis*. In this research, the liquid organic fertilizer used is Bio Ferti (Figure 1). Bio Ferti is a liquid organic fertilizer made from fermented vegetable and fruit waste with help of larva HI (*Hermetia illucens*). It contains some minerals functioning as enzymes such as phosphates, esterase (C4), esterase lipase, lipase, leucine arylamidase, valine arylamidase, and cysteine arylamidase (Kim et al. 2011). Bio Ferti also contains macronutrients such as N, P, K, Ca, Mg, and S around 103.771 mg/L (all value) and micronutrients such as Fe, Mn, Zn, and Cu around 0.2-0.62 mg/L (BPTP2007).

This study aimed to replace inorganic fertilizer with liquid organic fertilizer and to determine the influence of fertilizer on the growth and dry weight (biomass) of *S. platensis*.



**Figure 1.** Liquid organic fertilizer Bio Ferti (source: Faculty of Biology UGM).

## MATERIALS AND METHODS

### Microorganism and Media

The material needed in this research was the microalga *Arthrospira* (*Spirulina*) *platensis*. The culture of *Spirulina platensis* was taken from Nogotirto Algae Park UGM which had previously been cultivated as a research culture stock. The liquid organic fertilizer used was Bio Ferti to be compared with the inorganic fertilizer consisting of urea, NPK,  $\text{Na}_2\text{SO}_4$ , and soda ash. For urea was taken as much as 0.05 g, NPK 0.03 g, 0.015 g, and soda ash 0.075 g (Source: Nogotirto Algae Park). The inorganic fertilizer needed was then weighed and dissolved in 1 L of water. The doses of each Bio Ferti and the inorganic fertilizer were 2, 4, 6, 8, and 10 mL or in percent volume/volume was 0.4, 0.8, 1.2, 1.6 and 2 % v/v. The other materials needed were bottles, lamps, pumps, straw, and cotton.

### Cultivation

The cultures were grown in 500 mL bottle volume. For the work volume, 350 mL of water and 100 mL cells of *Spirulina platensis* were inoculated with three replicates. The bottles were equipped with supporting tools such as straw and cotton. The bottles were continuously aerated using pumps and illuminated by lamps. The cultivation was applied for seven days with 24 hours of light.

### Water Quality Parameters

The parameters observed in the culture media were temperature, pH, and salinity (the optimum salinity was 20 ppt). Measurement of temperature and pH were carried out every day to determine the quality of *Spirulina platensis* cultivation media.

### Analytical and Statistical Procedures

Cell density measurement was done by sampling 0.1 mL of *Spirulina platensis* using a dropping pipette and dropping it on a hemocytometer put under a light microscope. The number of cells located on the four corners and at the

center was recorded and then the total number of the cells was calculated using Eq.1 (Kawaroe et al. 2015). The dry weight of cells was measured by taking 10 mL of each of the samples every day during cultivation to be filtered using filter papers. Afterward, the filter paper is heated in the oven to evaporate the water at temperature of 60 °C for approximately 1 hour and then weighed to find out the sample weight. The dry cell weight means the difference between the weight of microalga-containing filter paper and that of the empty one.

$$A = Nx \frac{25}{5} \times 10 \tag{1}$$

Where:

A: Cell density (cell/mL)

N: Observed number of cell (cell)

The kinetic study was done by synthesizing the differential equations for batch conditions. Two models, namely the Contois (Eq. 2) and the Haldane (Eq. 3) models were proposed to study the growth kinetics. The differential equations were solved numerically in pairs, and the corresponding kinetic constants were determined by minimizing the sum of squares of errors (SSE) between the calculated and experimental data of the organic matter concentrations as dry weight of cell (X) and substrate (s). The differential equation pairs would be each of Eq. 2 and Eq. 3 paired with Eq. 4.

$$\mu = \mu_{max} \frac{s}{KsX+s} \tag{2}$$

$$\mu = \mu_{max} \frac{s}{s+Ks+\frac{s^2}{K_1}} \tag{3}$$

$$\frac{ds}{dt} = -\frac{1}{Y_{x/s}} \frac{dX}{dt} \tag{4}$$

Where:

$\mu$  : specific growth rate of microalgae (hours<sup>-1</sup> or days<sup>-1</sup>)

$\mu_{max}$  : maximum growth rate of microalgae (hours<sup>-1</sup> or days<sup>-1</sup>)

$Ks$  : half saturation coefficient (mg/L)

$s$  : concentration of nutrients in the medium at time (mg/L)

$ds/dt$  : substrate degradation rate with time

$Y_{x/s}$  : yield of dry cell formation to substrate decrease

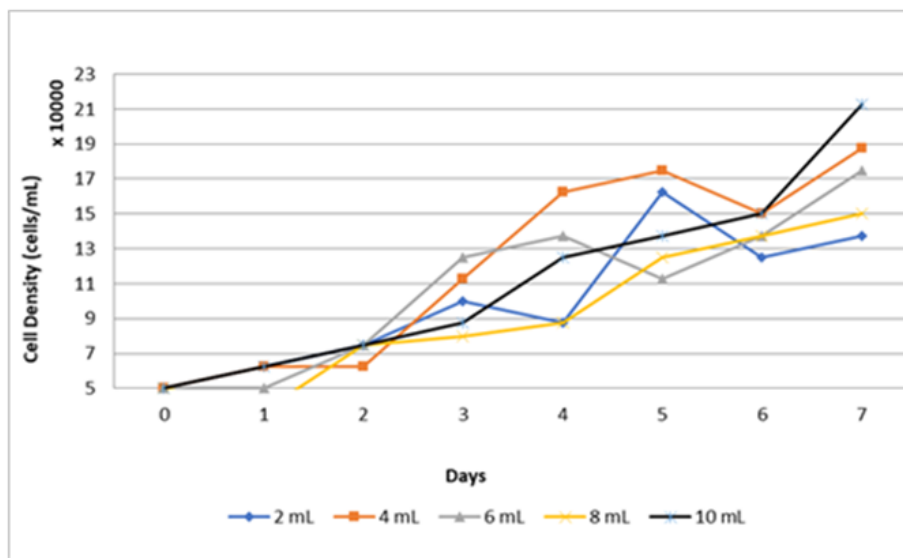
$dX/dt$  : dry cell formation rate with time.

## RESULTS AND DISCUSSION

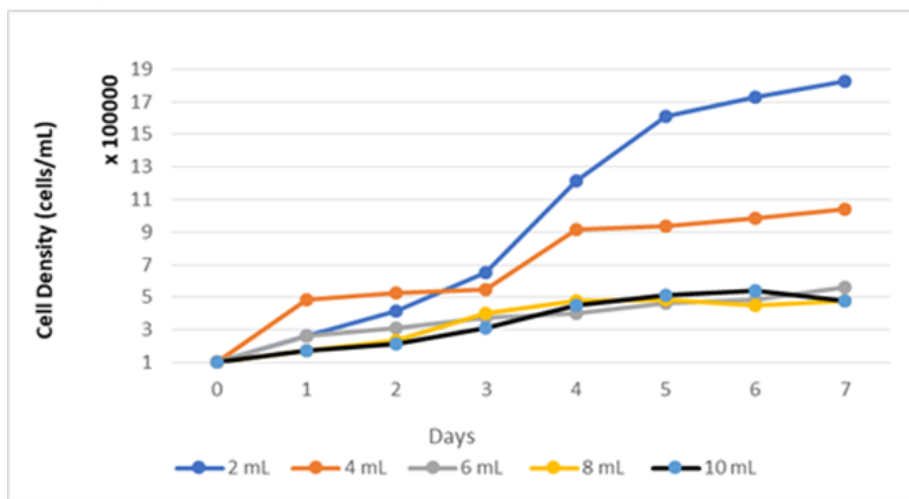
### *Spirulina platensis* Density

Microalgae growth is influenced by a variety of culture parameters, such as light intensity, pH, salinity, nutrients availability, temperature, CO<sub>2</sub>, and dissolved oxygen concentration (Chowdury et al. 2020). Microalgae growth consists of four phases, and those are the lag phase, exponential phase, stationary phase, and death phase. Nutrients are also needed by phytoplankton for growth. The density calculation of the *Spirulina platensis* was conducted to de-

termine the growth of *S. platensis*. This density calculation was conducted using a microscope and a hemocytometer as a tool to calculate the total density of *Spirulina platensis* cells. Observation was done for seven days for each concentration of fertilizer. From the daily results of the observed cell abundance, the peak time of *S. platensis* density can be discovered. Based on observation, due to differences in concentration in the used medium, the density of *S. platensis* cultured in each used medium was different. The results of the *S. platensis* cell density after using Bio Ferti liquid organic fertilizer and inorganic fertilizers are presented in Figures 2 (a and b).



a



b

**Figure 2.** Differences in cell density growth using (a) inorganic fertilizers and (b) Bio Ferti. The value is obtained by calculating using the cell density formula. This research was conducted using different nutrient concentrations, with qualitatively different results in each concentration.

The differences in the *S. platensis* density between using Bio Ferti and the inorganic fertilizer in this study are presented in Figures 2 (a and b). On the day after the doses were given, the density increased in the doses of 2, 4, and 6 mL. In Bio Ferti with the doses of 8 and 10 mL, it was stable or in oth-



er words it hasn't grown yet. Meanwhile, in the inorganic fertilizer, it decreased at the dose of 8 mL and was stable at the dose of 10 mL. On the second day after the doses were given, *S. platensis* in each treatment showed the ability to adapt to the culture media environment, except in the treatment using inorganic fertilizer, in which the growth was still slow. In the doses of 8 and 10 mL, the cells grew slowly because, at the time, it was at the adjustment phase to the culture media (Zahidah et al. 2012).

The growth of *S. platensis* during the cultivation is strongly influenced by the availability of nutrients, cell age, and light intensity in growing media (Sanchez-Saveedra & Voltolina 2005). Then, it was suspected that the factors inhibiting the growth of *S. platensis* in the adaptation phase in the dose of 8 mL using inorganic fertilizer were the equality of the inoculum used when stocking and the condition of culture media.

Based on Figures 2 (a and b), Bio Ferti with the dose of 2 mL produced the densest population of *S. platensis* on the 7<sup>th</sup> day with a density of 1,775,000 cells/mL, while the doses 4 and 6 mL reached it on the same day with densities of 987,500 and 512,500 cells/mL. The dose of 8 mL produced the densest population on the 5<sup>th</sup> day with a density of 437,500 cells/mL, and the dose of 10 mL reached it on the 6<sup>th</sup> day with a density of 487,500 cells/mL (Figure 2b).

Of the inorganic fertilizer, the doses of 2 mL produced the densest population on the 5<sup>th</sup> day with a density of 162,500 cells/mL, while the doses of 4, 6, 8, and 10 mL produced the densest population on the 7<sup>th</sup> day with densities of 187,500, 175,000, 150,000, and 2,122,500 cells/mL, respectively (Figure 2a).

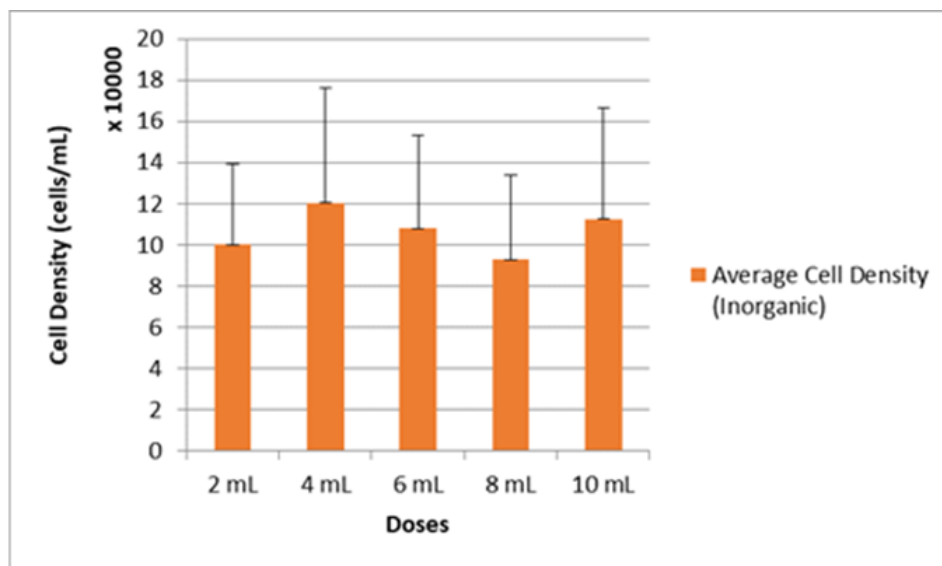
The experiments showed the highest density of 1,775,000 cells/mL was in the application of Bio Ferti with the dose of 2 mL. In the inorganic fertilizer, the highest density, namely 212,500 cells/mL, was shown by the dose of 10 mL (Figure 3).

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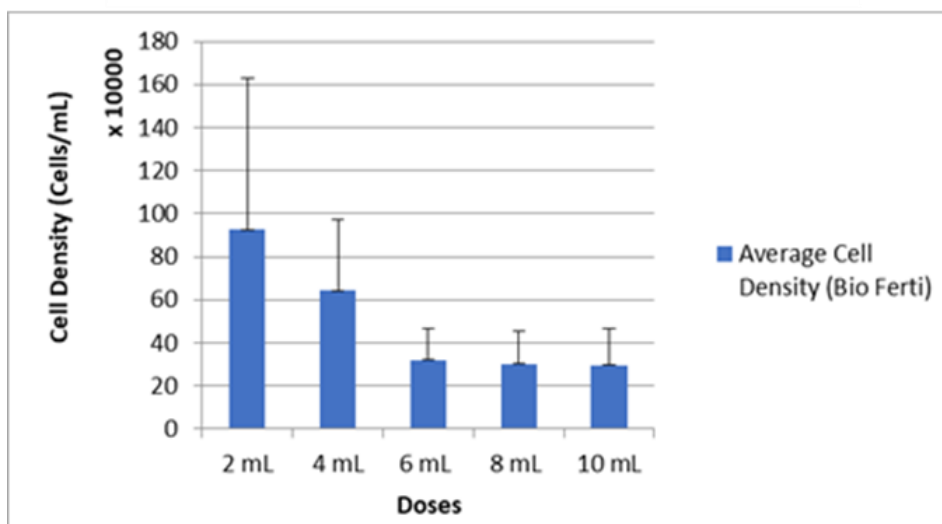
The lowest density in the application of Bio Ferti was shown by the dose of 10 mL, namely 425,000 cells/mL, while that in the application of inorganic fertilizer as indicated by the dose of 2 mL, namely 137,500 cells/mL (Figure 3). The high and low growth rates in the density of *S. platensis* cells were due to differences in nutrients in each treatment and the absorption of excess nutrients used for cell movement and growth processes.

The differences in cell density in each treatment show that *S. platensis* can use the nutrients contained in Bio Ferti and the inorganic fertilizer for its growth. One of the factors that influence the growth of *S. platensis* is the nutrients available, including macronutrients and micronutrients, which also are the factors that influence the biochemical composition of algae (Utomo et al. 2005). The differences in cell density are caused by the effect of differ-

ent doses of the organic fertilizer given. The quality of the nutritional contents of *S. platensis* is related to the composition of nutrients in the culture media and water quality parameters (Widianingsih et al. 2008).



a



b

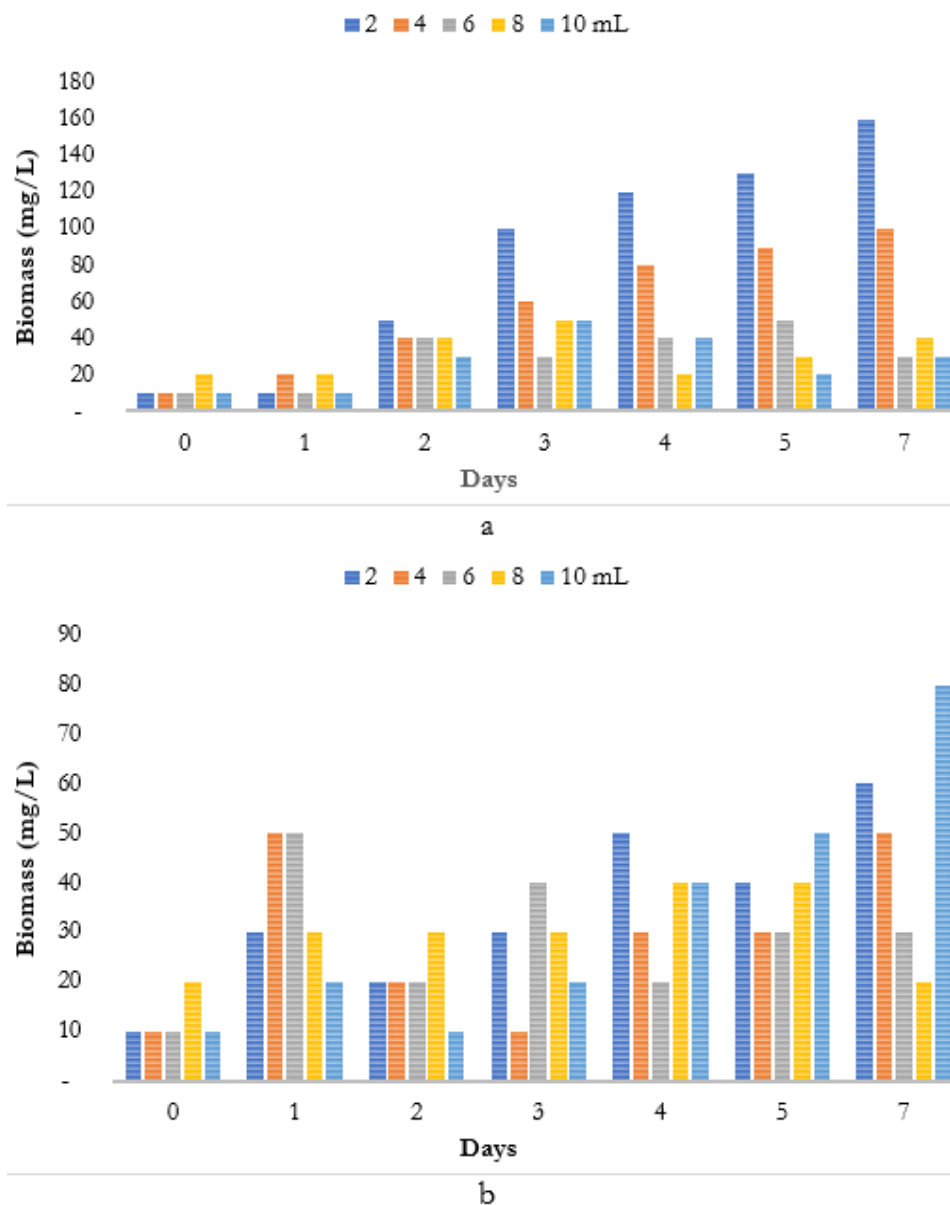
**Figure 3.** Average cell density of *Spirulina platensis* using (a) Inorganic fertilizer and (b) Bio Ferti. The value is obtained by calculating using the cell density formula. This research was conducted using different nutrient concentrations, with qualitatively different results in each concentration.

### Dry Weight (Biomass) of *Spirulina Platensis*

Dry cells are obtained from the yield of the growth of *Spirulina platensis* cells by assimilating the nutrient content contained in the medium into the cells. Based on the research, the biomass (dry cell) yield of *S. platensis* using Bio Ferti liquid organic fertilizer and inorganic nutrients are presented in Figures 4 (a and b).

The daily response of *Spirulina platensis* biomass to the inorganic fertilizer and Bio Ferti in this study is presented in Figures 4 (a and b). The increasing biomass in all media during the cultivation days showed different growth rates. There was no difference in Bio Ferti until the 1st day after the doses

were given. On the 2<sup>nd</sup> to the 7<sup>th</sup> days, the rate for the dose of 2 mL was higher than that of the other doses. The highest biomass achieved by Bio Ferti was 160 mg/L, with a dose of 2 mL, while that by the inorganic fertilizer was 80 mg/L with a dose of 10 mL. The decreasing rate of *S. platensis* biomass in each media was thought to be due to the depletion of nutrient availability and the increasing cell mass.



**Figures 4.** *Spirulina platensis* biomass in (a) Bio Ferti and (b) Inorganic Fertilizer. The dry cell weight value means the difference between the weight of microalga-containing filter paper and that of the empty one. This research was conducted using different nutrient concentrations, with qualitatively different results in each concentration.

The results of the analysis of some minerals contained in Bio Ferti showed that the lower the dose given, the more adequate the nutrients needed for *S. platensis* to grow. The higher the dose of Bio Ferti given; the less biomass of *S. platensis* produced. Meanwhile, for inorganic fertilizer, the higher the dose given, the higher the biomass produced. As mentioned above,



Bio Ferti contains N, P, K, Ca, Mg, and S around 103,771 mg/L. Several investigators reported that the growth and productivity of *S. platensis* were determined by phosphorus concentration in the cultivation media (Xin et al. 2010; Dammak et al. 2017). Meanwhile, nitrogen plays a role in the formation of macromolecules and cell biomass (Perez-Garcia et al. 2011). With a concentration of 2.5 g/L, it is optimum and recommended for cultivating *S. platensis* (Celekli & Yavuzatmaca 2009).

### Growth Kinetics

Based on the obtained result of the research, a dose of 2 ml is the optimum medium. Kinetic study was conducted at a dose of 2 ml to identify the growth kinetics of *Spirulina platensis* using two different mathematical models. The used models are the Contois model (Equation 2) and the Haldane model (Equation 3). This mathematical approach is used to predict the growth rate of microalgae and design microalgae mass culture. The numerical calculations were carried out using MATLAB.

The Contois and Haldane equations are corrected by considering another factor, and that is the death phase. The death phase factor is assumed to be one of the causes of the decrease in cell mass in microalgae culture.

$$\text{decay rate} = -K_d \cdot X \quad (5)$$

In a continuous system, the growth rate of microalgae can be determined by calculating the specific growth rate and concentration of microalgae.

$$\frac{dx}{dt} = \mu \cdot X \quad (6)$$

Thus, the Contois and Haldane equations in equations 2 and 3 become,

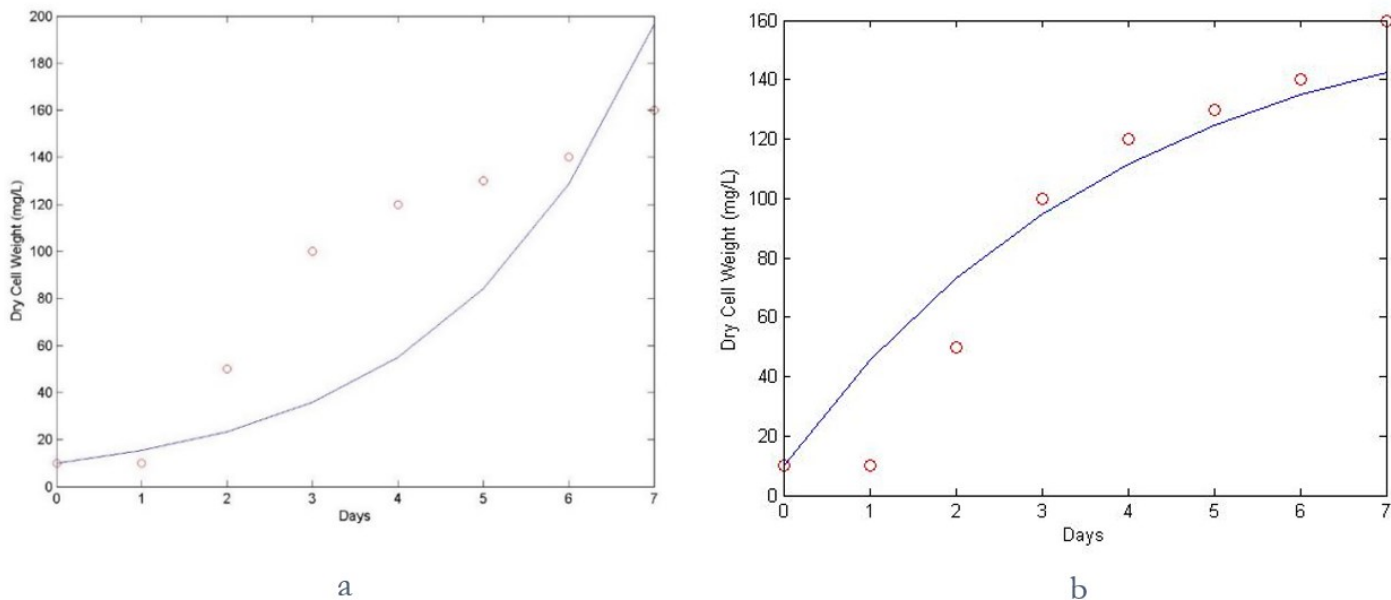
$$\frac{dx}{dt} = \mu_{max} \frac{s}{K_s X + s} X - k_d \cdot X \quad (7)$$

$$\frac{dx}{dt} = \mu_{max} \frac{s}{s + K_s + \frac{s^2}{K_1}} X - k_d \cdot X \quad (8)$$

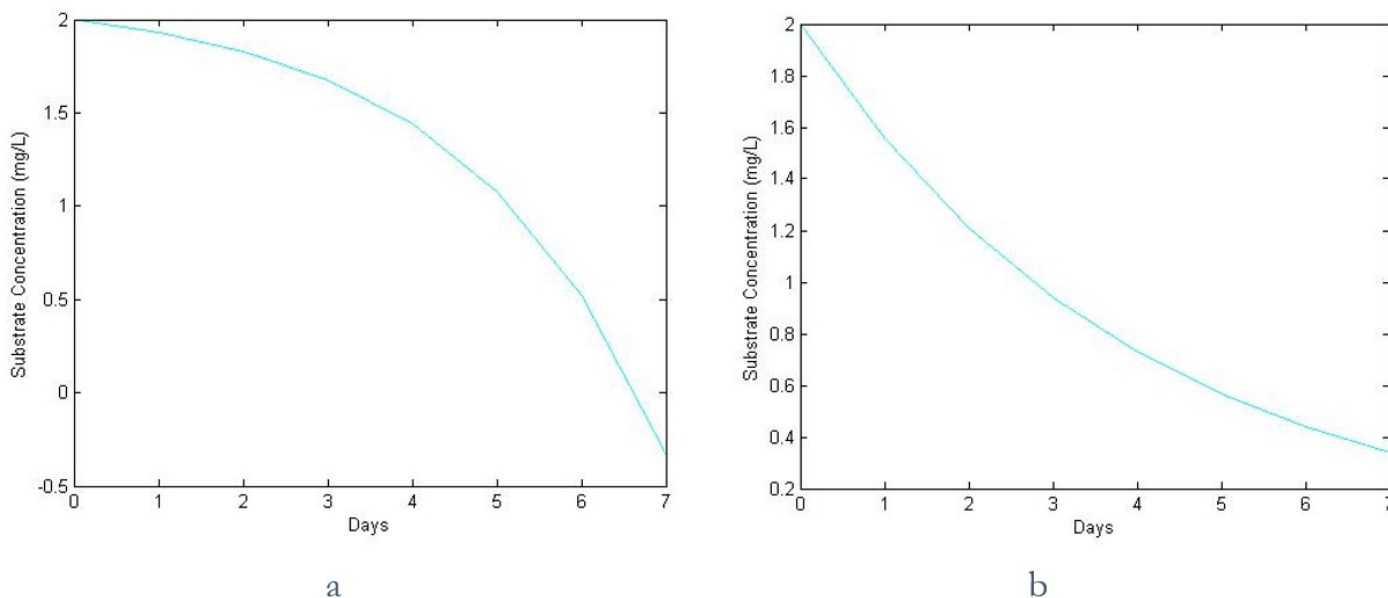
The results of the numerical simulation using the Contois and Haldane equations are presented in the graph of the relationship between experimental data and simulation data against time, a graph of the decrease in substrate during the cultivation process and the kinetics parameters of the microalgae *S. platensis* growth rate.

The result of the kinetic simulation using the Contois and Haldane models are presented in Figures 5 (a and b). The graph describes the relationship between dry cell weight and time period that shows a comparison of the estimated biomass to the experimental data. Based on the figure, there is a deviation that occurs in the dry cell weight curve. The significant deviation between the experimental and simulation data occurred on the second to the fifth day of each model.

Figures 6 (a and b) are curves of simulation results that show the relationship between substrate concentration and time period. Based on those



**Figures 5.** Comparison between experimental data and concentration estimates of dry cell weight using (a) the Contois and (b) the Haldane model. Blue line represents X simulation; Red line represents X experiment.



**Figures 6.** Comparison between substrate concentration and time period using (a) the Contois and (b) the Haldane models. Figure 6(a) and (b) are curves of simulation results that show the relationship between substrate concentration and time period.

two figures, there was a decrease in substrate concentration at a dose of 2 ml during the cultivation process. This occurred because the substrate was consumed by microalgae to reproduce and produce biomass and other components.

Quantitative analysis was conducted by looking at the parameter calculated from the mathematical modeling and kinetic simulation of the growth rate of *S. platensis* microalgae as presented in Table 1. The parameter values obtained from the simulation are maximum specific growth rate ( $\mu_{max}$ , day<sup>-1</sup>), half-saturation constant ( $K_s$ , mg/L), inhibition constant ( $K_i$ , mg/L), and death coefficient ( $K_d$  day<sup>-1</sup>).

From Table 1, the result of numerical calculations using MATLAB, the constant values generated in each model show different results. The value of the specific growth rate ( $\mu$ ) indicates the growth rate of *S. platensis* cells per unit of time. The kinetics of the growth rate was at its peak point in the exponential phase, the growth rate reached a maximum ( $\mu_{max}$ ) and the maximum density was reached. The  $\mu_{max}$  value obtained with the Contois model is 69.9558 day<sup>-1</sup>, while in the Haldane model of 187.2500 day<sup>-1</sup>, the low specific growth rate value describes the slow growth of microalgae.

**Table 1.** Kinetic constants of the two proposed models generated from MATLAB simulation.

Parameter	Model	
	Contois	Haldane
$\mu_{max}$	69.9558	187.2500
$K_s$	127.852	92.719
$K_d$	0.1169	0.01006
$K_I$	-	343.2452
SSE	126,530,000	22,695,000

The  $K_s$  value obtained in the Contois model is 127.852 mg/L, while in the Haldane model the value is smaller, which is 92.719 mg/L. Half-saturation constant ( $K_s$ ) describes the affinity of microorganisms for the substrate (Kim et al. 2003). The low  $K_s$  value using the Haldane model shows the result that microalgae have high efficiency or are faster in using substrates at low concentration. This can be seen in Figures 6 (a and b) where the substrate subsidence curve depicted by the Haldane Model is more positive than that of the Contois Model.

In determining the appropriate model to describe the growth kinetics of *S. platensis* microalgae culture, it was done by looking at the results of the lowest Sum of Squared Error (SSE) value. Sum of Squared Error is the total difference from experimental data to simulation data. The smaller SSE value indicates that the experimental data obtained are different from the data of the MATLAB simulation.

From Table 1, the SSE value generated using the Contois model is 126,530,000 while the SSE value obtained by the Haldane model is 22,695,000 which is smaller than the Contois model. Therefore, in this research, the appropriate model to study the growth kinetics of *S. platensis* at a dose of 2 ml is the Haldane model.

### Water Quality Parameters

Factors that influence the growth of *Spirulina platensis* cells during cultivation are water quality. Water quality parameters that were cultured as supporting factors for this research were temperature and degree of acidity (pH). Measurement of water quality parameters is carried out using digital tools. Each device has a sensor which will be immersed in the culture and then read the temperature and pH values on each screen.

### Temperature

Temperature is one of the factors that affect the growth of *S. platensis* cells. Measurement was made daily during cultivation. Temperature measurement ranged from 26-32 °C for each media. Changes in temperature during the research period were still within the tolerance limit, which still allows *S. platensis* to grow. The results of temperature measurements using Bio Ferti liquid organic fertilizer and inorganic fertilizer are presented in Table 2 and 3.

**Table 2.** Temperature data of *Spirulina platensis* using Bio Ferti liquid organic fertilizer.

Bio Ferti (mL)	Days							
	0	1	2	3	4	5	6	7
2	26.9	29.2	28.5	30.1	29.4	28.9	29.8	28.7
4	28.2	28.5	28.3	30.5	30.2	29.6	29.2	29.2
6	27.9	30.5	27.6	29.8	28.9	29.3	30.2	28.4
8	28.9	30.1	29.2	28.8	29.4	30.1	28.8	29.3
10	28.5	29.7	28.9	29.2	30.9	29.8	30.4	28.9

**Table 3.** Temperature data of *Spirulina platensis* using inorganic fertilizer.

Inorganic fertilizer (mL)	Days							
	0	1	2	3	4	5	6	7
2	30.2	28.5	30.5	31.4	30.2	29.8	29.3	30.3
4	31.2	30.5	30	30.4	31.9	30.6	29.7	29.2
6	31.5	29.5	31	32.4	31.2	31.8	29.8	30.5
8	30.9	30.2	31.5	31.8	30.9	31.4	29.2	30.6
10	31.8	30.4	31.3	31.9	31.6	30.8	29.3	30.3

Temperature directly affects the efficiency of photosynthesis and is a determining factor in the growth of *S. platensis*. In this research, it can be seen in Tables 2 and 3 that the temperature changes in each treatment during the cultivation period seemed to fluctuate. The temperature for the use of Bio Ferti liquid organic fertilizer ranged from 26-30°C. The temperature in the culture medium using Bio Ferti liquid organic fertilizer increased and decreased in each treatment with the highest temperature 30.5°C and the lowest temperature of 26.8°C, while for the use of inorganic fertilizer the temperature value ranged from 28-31°C, with the highest temperature concentration of 31.5°C and the lowest of 28.5°C. Normally in laboratory condition, temperature change is influenced by room temperature and light intensity.

The optimum temperature range for the growth of *S. platensis* is 20-30°C (Hariyati 2008). The temperature changes in this research were still within the optimum temperature range for the growth of *S. platensis*. According to Odum (1973), although the temperature doesn't vary in water as much as in air, temperature is one of the limiting factors for aquatic organisms, condition below or above the optimum temperature will result in inhibition of organism growth and development. In fact, at extreme temperatures, organisms

will die (Wahyudi 1999). Thus, keeping the temperature of the culture medium in optimum condition is necessary for the optimum growth of *Spirulina platensis*.

### PH Value

The acidity degree (pH) is one of the important factors aside from temperature. pH measurement was conducted every day for seven days of the cultivation. The results of the pH measurements are presented in Tables 4 and 5.

pH is the solubility of minerals in the culture medium and directly affects the metabolism of microorganisms (Borowitzka1988). According to (Prihantini et al. 2005), drastic changes in pH can affect the work of enzymes and can inhibit the photosynthesis process and the growth of microalgae. Based on the table, from the pH measurement in each medium the pH values ranging from 8-9 are obtained. The pH value is still within the tolerance limit for *S. platensis* growth media because one of the characteristics of this alga is that it can live in slightly alkaline water condition. Cifferi (1983) stated that optimum pH for the growth of *S. platensis* ranged from 7-11. Blue-green algae grow well at neutral pH and tolerate more alkaline than acidic conditions because algae can utilize carbon dioxide efficiently although it is available at a very low concentration (Hariyati 2008).

**Table 4.** pH data of *Spirulina platensis* using Bio Ferti liquid organic fertilizer.

Bio Ferti (mL)	Days							
	0	1	2	3	4	5	6	7
2	8.01	8.30	8.26	8.27	8.27	8.26	8.50	8.22
4	8.08	8.26	8.24	8.27	8.29	8.31	8.30	8.17
6	8.11	8.30	8.25	8.28	8.28	8.26	8.40	8.18
8	8.11	8.33	8.26	8.29	8.26	8.28	8.40	8.18
10	8.15	8.31	8.26	8.28	8.27	8.30	8.40	8.2

**Table 5.** pH data of *Spirulina platensis* using inorganic fertilizer.

Inorganic fertilizer (mL)	Days							
	0	1	2	3	4	5	6	7
2	8.5	8.67	8.53	8.85	8.89	8.83	8.08	8.42
4	8.53	8.7	8.91	8.78	8.65	8.67	8.05	8.46
6	8.47	8.71	8.51	8.58	8.51	8.71	8.13	8.38
8	8.56	8.7	8.6	8.67	8.85	8.52	8.11	8.36
10	8.49	8.85	9.02	8.75	8.61	8.48	8.06	8.18

### CONCLUSION

The conclusion of this research showed that liquid organic fertilizer Bio Ferti can be used as an alternative to commercial fertilizers and inorganic fertilizers because the resulting production is higher in cell density and dry weight. The 2 ml dose is the optimum dose with the highest growth rate and highest cell density. The highest cell density was on day 7 which was  $1.78 \times 10^6$ . The appropriate growth kinetics model to study the growth kinetics of *Spirulina platensis* at the optimum dose of 2 ml is the Haldane model. It yielded a maxi-

imum specific growth rate of 187.2500 day<sup>-1</sup>, a half-saturation constant of 92.719 and SSE value of 22,695,000. Therefore, in this research, the appropriate model to study the growth kinetics of *S. platensis* at a dose of 2 ml is the Haldane model.

### AUTHORS CONTRIBUTION

MIMG conducted the research and the data analysis and wrote the manuscript, EAS, MDK, and LTS supervised the research and manuscripts writing, while UJS and AB supervised and designed the research.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publishing of this article. The authors certify that the manuscript is original work and is not under review at any other publication.

### REFERENCES

- Andrade et al., 2009. Culture of micoralga *Spirulina platensis* in alternative sources of nutrients. *Cienc.Agrotec*, 32(5), pp.1551-1535.
- Balai Pengkajian Teknologi Pertanian (BPTP), 2007, Utilization of Vegetable and Fruit Waste as Liquid Organic Fertilizer and Animal Feed, viewed on 22 August 2021 from <https://jakarta.litbang.pertanian.go.id/ind/index.php/hasil-pengkajian/pertanian/96-pemanfaatan-limbah-sayuran-dan-buah-buahan-sebagai-pupuk-organik-cair-dan-pakan-ternak.html>.
- Bandara, J. & Arunakumara, K., 2020. Development of low-cost growing media for *Spirulina platensis* using alternative carbon resources. *Vidyodaya Journal of Science*, 24, pp.41-47.
- Borowitzka, M.A., 1998. *Lipid Metabolism in The Red Marine Algae Chondrus Cripusand Polysiphonza Lanosa as Modified by Temperature*, United Kingdom: School of Bioscience, Cardif University.
- Celekli, A. & Yavuzatmaca, M., 2009. Predictive modelling of biomass production by *Spirulina platensis* as a function of nitrate and Nacl concentrations. *Biores Tech*, 100, pp.1847-1851.
- Chowdury et al., 2020. The Growth Factors Involved in Microalgae Cultivation for Biofuel Production: A Review. *Computational Water, Energy, and Environmental Engineering*, 9, pp.185-215. doi:10.4236/cweee.2020.94012



- Cifferi, O., 1983. *Spirulina* The Edible Microorganism. *Microbiological Reviews*, 47(4), pp.551-578.
- Dammak et al., 2017. Effects of nutritional conditions on growth and biochemical composition of *Tetraselmis* sp. *Lipids Health Dis*, 16, p.41. <http://dx.doi.org/10.1186/s12944-016-0378-1>.
- Gouveia, L. & Oliveira, A.C.J., 2009. Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, 36, pp.269-274.
- Guerin et al., 2003. *Haematococcus* astaxanthin: applications for human health and nutrition. *Trends Biotechnol*, 21(5), pp.210-216.
- Hariyati, R., 2008. Growth and Biomass of *Spirulina* sp in a Laboratory Scale. *Biological Scientific Periodic*, 10(1), pp.19-22.
- Hu, Q., 2004. Industrial production of microalgal cell-mass and secondary products-major industrial species: *Arthosphora (Spirulina) platensis*, In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp. 264-272.
- Ilknur, A.K., 2011. Effect of an organic fertilizer on growth of blue-green alga *Spirulina platensis*. *Aquaculture international*, 20, pp.413-422. doi:10.1007/s10499-011-9473-5.
- Kawaroe et al., 2015. Chemical mutagenesis of microalgae *Nannochloropsis* sp using EMS (*Ethyl Methanesulfonate*). *British Journal of Applied Science and Technology*, 8(5), pp. 1038-1045.
- Kim et al., 2003. Kinetics of benzene biodegradation by *Pseudomonas aeruginosa*: parameter estimation. *Environmental Toxicology Chemistry*, 22(5), pp. 1038-1045.
- Kim et al., 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology*, 14, pp.11-14.
- Li, Y et al., 2008. 'Biofuels from microalgae', *Biotechnology Progress*, vol.24, pp.815-820
- Madkour et al., 2012. Production and nutritive value of *Spirulina platensis* in reduced cost media. *The Egyptian Journal of Aquatic Research*, 38, pp.51-57.
- Mezzomoet al., 2010. Cultivation of microalgae *Spirulina platensis* from biological treatment of swine wastewater. *J. Technol*, 1, pp.173-178.
- Odum, E.P., 1973. *Fundamentals of Ecology*, Yogyakarta: Gadjah Mada University Press.
- Perez-Garcia et al., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. *Water Res*, 45, pp.11-36.
- Pioreck et al., 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry*, 23(2), pp.207-216.
- Prihantini et al., 2005. *Growth of Chlorella sp. in Medium Bean Extract (MET) With Variation of Initial PH*. Department of Biology, Depok: Faculty of Mathematics and Natural Sciences, University of Indonesia.

- Sanchez-Saveedra, M.P. & Voltolina, D., 2005. The growth rate, biomass production and composition of *Chaetoceros* sp Grown with different light sources. *Aquaculture engineering journal*, 35, pp.161-165.
- Usharani et al., 2012. *Spirulina* cultivation: A review. *International Journal of Pharmaceutical and Biological Archiev*, 3, pp.1327-2341.
- Utomo et al., 2005. *The growth of Spirulina platensis cultured with inorganic fertilizer (Urea, TSP, dan ZA) and chicken manure*. Bogor: Majoring aquaculture, faculty of fisheries and machine science, IPB University.
- Wahyudi, P., 1999., *Chlorella: Microalgae Source of Single Cell Protein*. Indonesian *Journal of Science and Technology*, 1(5), pp.35-41.
- Walach et al., 1987. Computer control of carbon-nitrogen ratio in *Spirulina platensis*. *Biotechnol Bioeng*, 29(4), pp.520-528.
- Widianingsih et al., 2008. Nutritional content of *Spirulina platensis* cultured on different media. *Indonesian Journal of Marine Sciences*, 13(3), pp.167-170.
- Xin et al., 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a fresh water microalga *Scenedesmus* sp. *BioresourTechnol*, 101(14), pp.5494-5500.
- Zahidah et al., 2012. The growth of *daphnia* sp populations fed with floating net karamba aquaculture fertilizer in fermented cirata reservoir EM4. *Journal Aquatica*, 3(1), pp.84-89.