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# Optimization of Spirulina sp. Growth in Walne Media with Variation of Urea and NaHCO<sub>3</sub> Supplements

[Caturwati, Lusiana Nova](#)  ; [Setyati, Retno Herrani](#)
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One alternative biofuel to substitute fossil fuels is bioethanol. Microalgae *Spirulina* sp. contains high carbohydrates, which has 17-25% potential to produce bioethanol. Urea and NaHCO<sub>3</sub> can be used as additional nutrients sources of nitrogen and carbon to *Spirulina* sp. cultivation. Deficiency of nitrogen causing the cell's enzymes change that shown through decreased lipid and chlorophyll synthesis. While deficiency of carbon can affect the growth rate. In this research, the growth rate of *Spirulina* sp. is analyzed using Optical Density (OD) method. The growth rate calculation is used to measure the growth of microalgae cells shown in the growth curve. This was a laboratory-scale method using CRD with 4 treatments and 5 replications namely treatment A addition of 0.36 g/500 ml urea without addition of NaHCO<sub>3</sub>, treatment B addition of 0.043 g/500 ml NaHCO<sub>3</sub> without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml NaHCO<sub>3</sub>, and control without addition of urea or NaHCO<sub>3</sub>. The results indicated that addition of urea and NaHCO<sub>3</sub> didn't affect to OD and *Spirulina* sp. growth rate. The highest growth rate was treatment A with 0.00906/day of growth rate followed by treatment C which has 0.00865/day of growth rate. Treatment B and control treatment (K) showed a low growth rate. The maximum OD value obtained in treatment C was 0.674 cells/ml on the 10th day. This research can be used as the reference to larger scale of *Spirulina* sp. cultivation in the field of bioethanol production. © 2020, J. Tropical Biodiversity Biotechnology

**Author keywords**Cultivation; Growth rate; Optical density (OD); *Spirulina* sp

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
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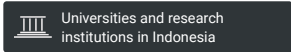




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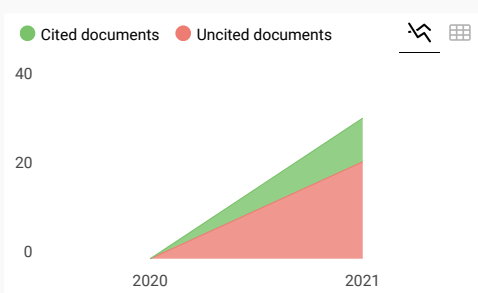
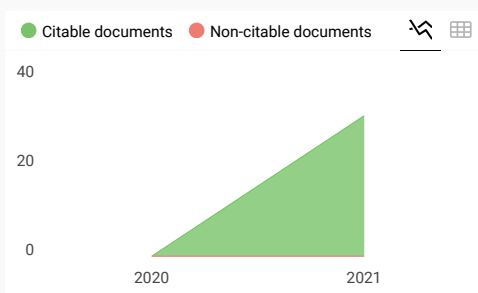
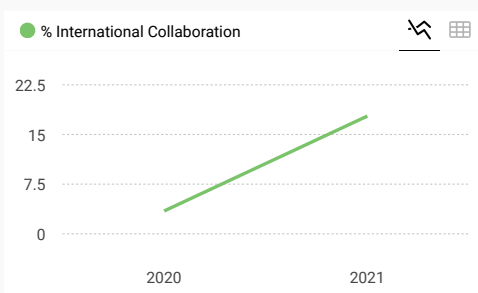
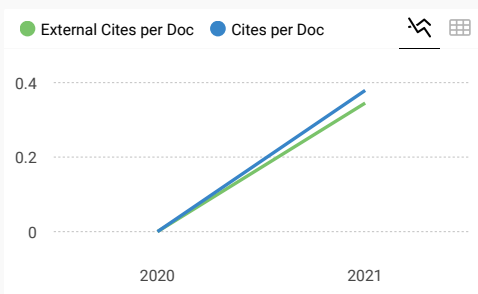
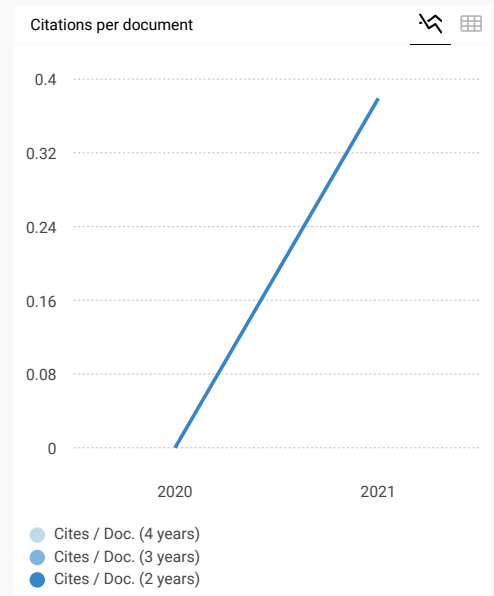
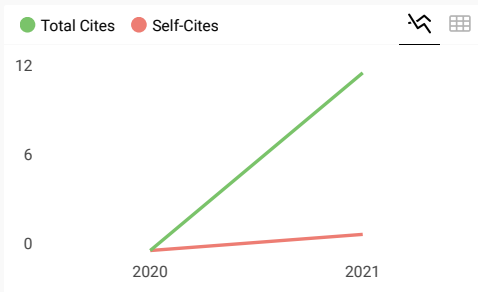
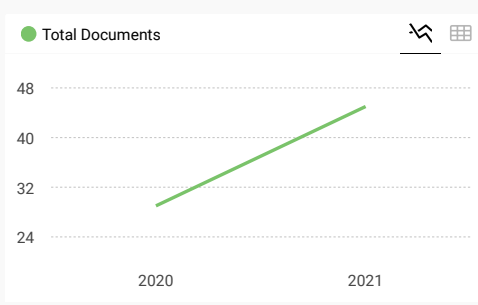
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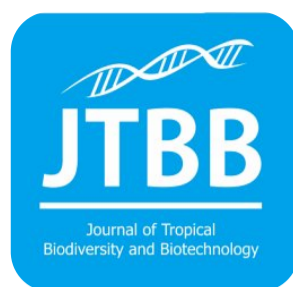
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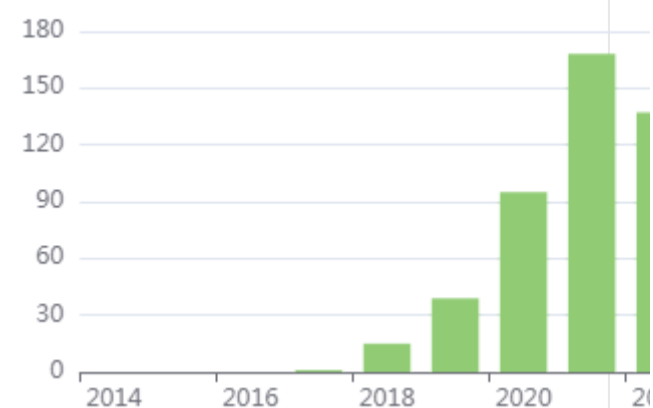
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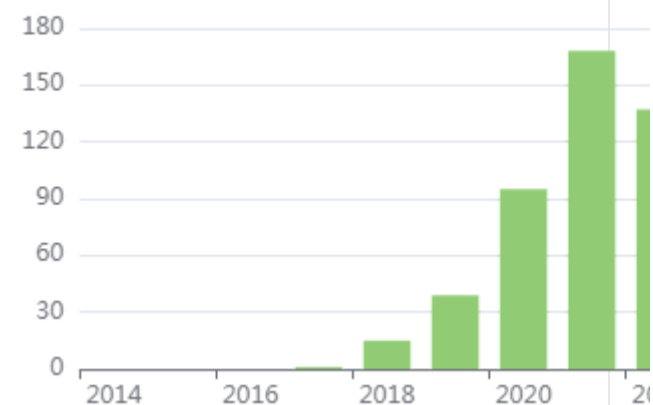
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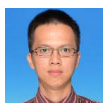
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Menu

Home About Login Register Search Current Archives Announcements Statistics Indexing & Accreditation Sitemap Contact

Home > Archives > Vol 5, No 1 (2020)

## Vol 5, No 1 (2020)

April

### Full Issue

[View or download the full issue](#)

### Table of Contents

#### Short Communications

Bioethanol Levels of Dragon Fruit (*Hylocereus polyrhizus*) Peel with the Addition of Blend Crude Cellulase Enzyme from *Trichoderma reesei* and *Aspergillus niger* 1-5

*Trianik Widyaningrum, Masreza Parahadi*

 [10.22146/jtbb.52189](https://doi.org/10.22146/jtbb.52189)  Abstract views : 2001 |  views : 1457

Diversity and Distribution of Herpetofauna in Banyu Nibo Waterfall Ecotourism Region, Nglangeran, Gunung Kidul, Special Region of Yogyakarta 6-9




*Alfonsus Toribio Eko Saputro, Elika Boscha, Ananto Puradi Nainggolan, Donan Satria Yudha, Rury Eprilurahman*

 [10.22146/jtbb.53637](https://doi.org/10.22146/jtbb.53637)  Abstract views : 1810 |  views : 1934

#### Research Articles

Vegetation Composition of Savanna Ecosystem as a Habitat For The Komodo Dragon (*Varanus komodoensis*) on Padar and Komodo Islands, Flores East Nusa Tenggara Indonesia 10-15

*S. Sutomo*

 [10.22146/jtbb.48280](https://doi.org/10.22146/jtbb.48280)  Abstract views : 2723 |  views : 3271

Conservation, Phytoremediation Potential and Invasiveness Status of Bali Botanic Garden Aquatic Plant Collection 16-26

*Farid Kuswanto*

 [10.22146/jtbb.49312](https://doi.org/10.22146/jtbb.49312)  Abstract views : 147239 |  views : 1763

Tree Species Composition and Natural Regeneration Status of Southeast Region of Bangladesh 27-34

*Abir Dey, Aklima Akther*

 [10.22146/jtbb.49988](https://doi.org/10.22146/jtbb.49988)  Abstract views : 1981 |  views : 1658

Characteristics of Shell Bone as an Identification Tool for Turtle Species (Reptiles: Testudines) in Java, Borneo, and Sumatra 35-43

*Donan Satria Yudha, Fidelis Triki Sadewa Aritona, Rury Eprilurahman*

 [10.22146/jtbb.47227](https://doi.org/10.22146/jtbb.47227)  Abstract views : 2726 |  views : 1760

Diversity analysis of *Rhacophorus margaritifer* (Schlegel, 1837) in Baturraden based on RAPD markers 44-52

Sitemap Contact

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JOURNAL CONTENT

Search




Fajar Nur Sulistyahadi, I Gusti Agung Ayu Ratna Puspitasari, Agus Nuryanto

 10.22146/jtbb.49110  Abstract views : 1439 |  views : 1500

Optimization of *Spirulina* sp. Growth in Walne Media with Variation of Urea and NaHCO<sub>3</sub> Supplements

53-58




Lusiana Nova Caturwati, Retno Herrani Setyati

 10.22146/jtbb.53635  Abstract views : 2655 |  views : 2417

Effect of *Arthrospira maxima* and *Chlorella vulgaris* to Lipid Profile and Visceral Fat Index Alteration in Streptozotocin-Induced Hyperglycemia Rats

59-67




Mulyati Mulyati, Aprilia Rahmawati, Slamet Widiyanto

 10.22146/jtbb.49937  Abstract views : 1588 |  views : 1373

RAPD Analysis for Genetic Variability Detection of Mutant Soybean (*Glycine max*(L.) Merr)

68-77

Didik Wahyudi, Lia Hapsari, S. Sundari

 10.22146/jtbb.53653  Abstract views : 2552 |  views : 2058

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## Research Article

# Optimization of *Spirulina* sp. Growth in Walne Media with Variation of Urea and NaHCO<sub>3</sub> Supplements

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### ABSTRACT

One alternative biofuel to substitute fossil fuels is bioethanol. Microalgae *Spirulina* sp. contains high carbohydrates, which has 17-25% potential to produce bioethanol. Urea and NaHCO<sub>3</sub> can be used as additional nutrients sources of nitrogen and carbon to *Spirulina* sp. cultivation. Deficiency of nitrogen causing the cell's enzymes change that shown through decreased lipid and chlorophyll synthesis. While deficiency of carbon can affect the growth rate. In this research, the growth rate of *Spirulina* sp. is analyzed using Optical Density (OD) method. The growth rate calculation is used to measure the growth of microalgae cells shown in the growth curve. This was a laboratory-scale method using CRD with 4 treatments and 5 replications namely treatment A addition of 0.36 g/500 ml urea without addition of NaHCO<sub>3</sub>, treatment B addition of 0.043 g/500 ml NaHCO<sub>3</sub> without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml NaHCO<sub>3</sub>, and control without addition of urea or NaHCO<sub>3</sub>. The results indicated that addition of urea and NaHCO<sub>3</sub> didn't affect to OD and *Spirulina* sp. growth rate. The highest growth rate was treatment A with 0.00906/day of growth rate followed by treatment C which has 0.00865/day of growth rate. Treatment B and control treatment (K) showed a low growth rate. The maximum OD value obtained in treatment C was 0.674 cells/ml on the 10th day. This research can be used as the reference to larger scale of *Spirulina* sp. cultivation in the field of bioethanol production.

**Keywords:** Cultivation, *Spirulina* sp., growth rate, optical density (OD).

### INTRODUCTION

As the time flies, biofuel is highly needed for transportation and industry. The fuel that is often used to fulfill human needs is fossil fuel that can't be renewed such as coal, natural gas, and petroleum. In fact, its availability in nature is decreasing. Meanwhile, using biofuel as alternative energy can replace the use of fossil fuel.

The alternative energy is produced from other resources that will not run out and sustainable if it is managed properly. One of the biofuels called bioethanol, made from fermented sugar liquid from carbohydrate source by using microorganisms. Bioethanol developed from wastes that still contains carbohydrate (Seftian *et al.* 2012). According to Dewi (2016), bioethanol can be produced from variety of plants such as corn, cassava, potatoes, sugar cane,

sorghum, and algae.

Microalgae is a potential energy source in the future because it contains carbohydrates that can be processed into several types of compounds such as biodiesel, bioethanol, and methane (Melanie and Diini, 2015; Hadiyanto and Maulana, 2012). Microalgae is an unicellular organism. It has chlorophyll and utilizes the process of photosynthesis to produce biomass. Microalgae is widely used to produce food supplements because of its high protein. The advantages of microalgae compared to other organisms are able to produce biomass and energy supplies in a short time, only need a small area, can be grown on non-productive land, and high growth rate so it is easily to cultivate (Hadiyanto and Maulana, 2012).

*Spirulina* sp. is a type of microalgae with carbohydrates and high protein. According to Christwardana *et al.* (2012), *Spirulina* sp. contains about 17-25% of carbohydrate and 56-62% of

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protein. The high content of carbohydrate from this microalga cause *Spirulina* sp. potentially produce bioethanol as a renewable energy product. The advantages possess by *Spirulina* sp. from other types of microalgae are relatively fast to reproduce and easier to harvest because it has a large biomass size (Syaichurozzi and Jayanudin, 2016). Cultivation of *Spirulina* sp. can use sea water, fresh water, or brackish water. Cultivation requires appropriate culture media and contains nutrients for *Spirulina* sp. *Spirulina* sp. also requires additional nutrients both macro and micro nutrients for their survival. Those macro nutrients are N, P, C, H, O, Ca, Mg, Na, and K, while the micro nutrients are Fe, Mn, Cu, Zn, B, and cyanocobalamin (Sari *et al.*, 2012).

According to Widianingsih (2008), carbon (C) and nitrogen (N) are the most important elements for *Spirulina* sp. to growth. The carbon is used in respiration, an energy source, and raw material for the formation of additional cells. The lack of carbon in the growth media affects the growth rate. Nitrogen play a role in the formation of proteins and nucleic acid. Ambarwati *et al.* (2018), also say that nitrogen play a role to stimulate vegetative growth and increase cell number. The deficiency of nitrogen can cause the enzymes in cells change that shown through decreased lipid synthesis and chlorophyll synthesis (Juneja *et al.*, 2013). Urea and  $\text{NaHCO}_3$  can be used as additional nutrients to fulfill the survival of *Spirulina* sp. Urea is a source of nitrogen (N) while  $\text{NaHCO}_3$  is a source of carbon (C). Urea and  $\text{NaHCO}_3$  are more economical and easier to obtain than other N and C sources.

The maximum density of *Spirulina* sp. can be seen through the measurement of OD (Optical Density) on growth media. OD value is proportional to the population density of microalgae. Density measurement used to analyze the growth rate of microalgae using OD method. According to Prayitno (2006), the calculation of growth rate used as a measure growth speed of microalgae cells. The results of OD measurements during this cultivation period can be shown through a microalgae curve. The growth curve used as a determinant when microalgae enter the highest density peak. The application of urea and  $\text{NaHCO}_3$  are mixed in a *Spirulina* sp. medium at certain doses. One of the growth media of *Spirulina* sp. is Walne's media. According to Widianingsih (2008), Walne's media is a good culture media for *Spirulina* sp. growth. This research is to determine the effect of urea and  $\text{NaHCO}_3$  supplies on OD and the growth rate of *Spirulina* sp., to find out the value of *Spirulina* sp.growth rate with urea and  $\text{NaHCO}_3$  supplements, and to find out the maximum OD value of *Spirulina* sp.

## MATERIALS AND METHODS

This was a laboratory scale method using Completely Randomized Design (CRD) with 4 treatments and 5 replications namely; treatment A addition of 0.36 g/500 ml urea without addition of  $\text{NaHCO}_3$ , treatment B addition of 0.043 g/500 ml  $\text{NaHCO}_3$  without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml  $\text{NaHCO}_3$ , and control without addition of urea or  $\text{NaHCO}_3$ . Microalgae used in this study was *Spirulina* sp. from Balai Pengembangan Teknologi Perikanan Budidaya (BPTPB) Cangkringan, Yogyakarta. This study used Walne media as basic media. Cultivation was carried out for 10 days with batch culture method. The standardization of *Spirulina* sp. cells at the beginning of cultivation cannot be uniform, so the standardization in this study is the volume of inoculants and the volume of media. Optical density (OD) of *Spirulina* sp. measured every 24 hours for 10 days cultivation using Spectrophotometer UV-1800 Shimadzu wavelength 680 nm according to the research of Syaichurozzi and Jayanuddin (2016). The volume of sea water as a solvent of Walne media is 300 ml, volume inoculant 200 ml, Walne media, and vitamin  $\text{B}_{12}$  addition respectively 0.3 ml, pH 8.5, salinity 27‰, aeration, and lighting every day for 24 hours.

*Spirulina* sp. cultivation tools used 500 ml culture bottles, aerator, two 40-watt tube light lamps on each rack, and aeration hose arranged as in Figure 1. The cultivation tools were designed in a such way to adjust BPTPB Cangkringan laboratory conditions, which was the place to do the research.

Researchers used the formula presented by Hirata *et al.* as referred by Kawaroe *et al.* (2009); Kawaroe *et al.* (2015); and Syaichurozzi & Jayanudin (2016) which uses the same formula to calculate the growth rate as follows:

$$k = \frac{\log \frac{N_1}{N_0}}{T_1 - T_0} \times 3.22$$

Where:

- k = growth rate(/day)
- $N_1$  = microalgae density at time t
- $N_2$  = microalgae density at time 0
- 3.22 = constant
- $T_1$  = observation time at time t
- $T_0$  = observation time at time 0

Data analysis was performed by quantitative descriptive methods and simple statistics by calculating the average value of Optical Density (OD) to get the growth rate and curve making. Data analysis technique used to obtain the most optimal nutrient addition for *Spirulina* sp. conducted

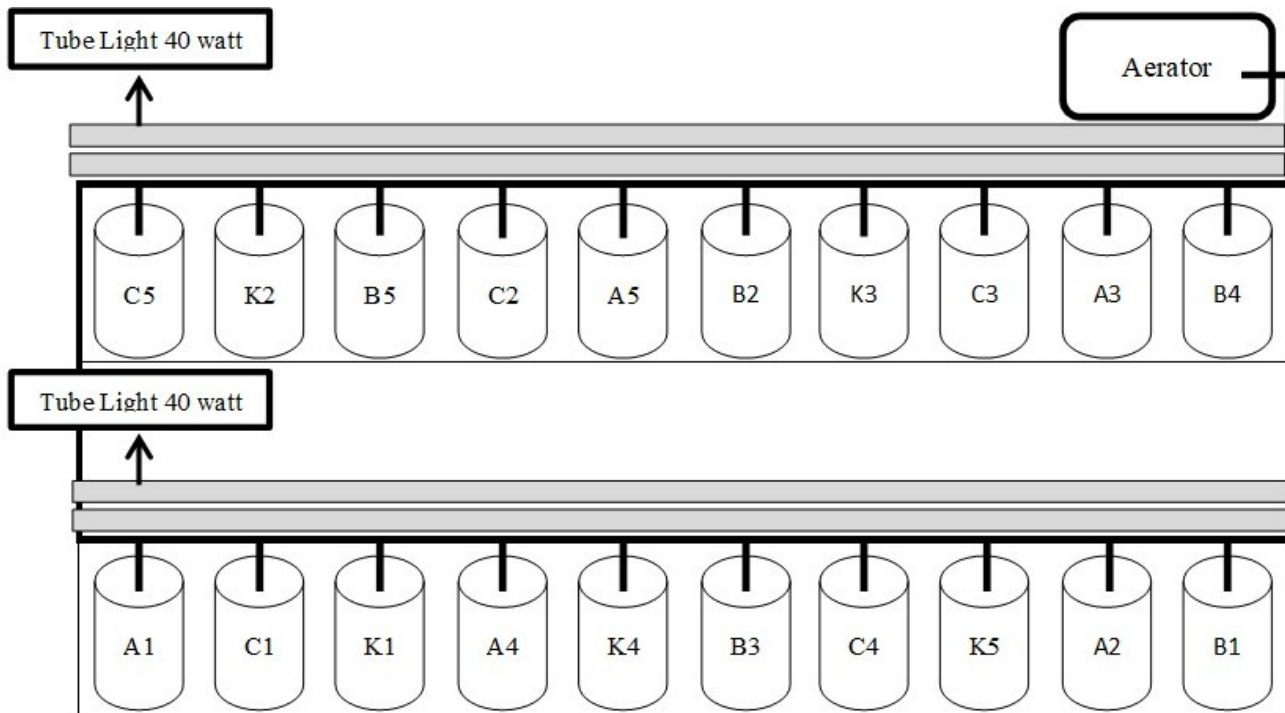


Figure 1. Design of cultivation tools.

quantitatively using the ANOVA test on IBM SPSS Statistics version 20. In this study, the X variable is the addition of urea and NaHCO<sub>3</sub>, while the Y variable represents the Optical Density (OD) value. Before the ANOVA test, normality tests and homogeneity tests were carried out to ensure that the data are normal and homogeneous.

## RESULTS AND DISCUSSION

### The Density and Growth Pattern of *Spirulina* sp.

Density data were calculated starting from the 0<sup>th</sup> day of cultivation to the 10<sup>th</sup> day. Based on Figure 2, it's shown that the highest density value on treatment K (control) was on the 6<sup>th</sup> day which was 0.492 cells/ml, treatment A on the 10<sup>th</sup> day which was 0.672 cells/ml, treatment B on the 7<sup>th</sup> day which was 0.494 cells/ml, and treatment C on the 10<sup>th</sup> day which was 0.674 cells/ml. The density of *Spirulina* sp. at the beginning cannot be formed so that it has a different value in each treatment. However, differences in density are not very different from one another.

Increased density on day 0 to 1<sup>st</sup> day indicates that *Spirulina* sp. has adapted to new growth media. Suyono and Winarto (2006), mentioned that in the process of microalgae cell adaptation, they have utilized the nutrients in the media, although not yet optimal. This phase is called the lag phase (induction phase). Muyassaroh (2018) also stated that the growth of *Spirulina* sp. marked with a bluish-green

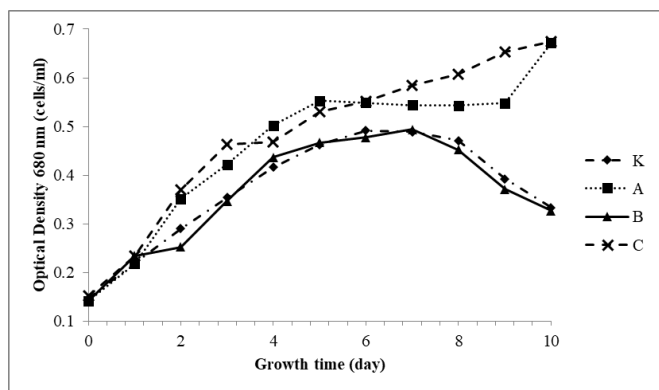
color in the growth media, while a yellowish color in the growth media shows that *Spirulina* sp. has experienced a phase of death. The thicker bluish greencolor on the media shows that the growth of *Spirulina* sp. increased both in terms of size and number of cells.

Based on the growth curve from Figure 2, it can be seen that the growth of *Spirulina* sp. each treatment has a different pattern. The density of *Spirulina* sp. the treatment K (control) continued to increase since the first day of cultivation and experienced a peak on the 6<sup>th</sup> day, but decreased from the 7<sup>th</sup> day to the 10<sup>th</sup> day. The density of *Spirulina* sp. treatment A continued to increase from the 1<sup>st</sup> day of cultivation to the 5<sup>th</sup> day, then down to the 9<sup>th</sup> day, and increased again on the 10<sup>th</sup> day. The density of *Spirulina* sp. treatment B continued to increase since the 1<sup>st</sup> day of cultivation and experienced a peak on the 7<sup>th</sup> day, then decreased from the 8<sup>th</sup> to the 10<sup>th</sup> day, while the density of *Spirulina* sp. treatment C has increased since the 1<sup>st</sup> day of cultivation and continues to increase until the 10<sup>th</sup> day. In treatments A and C, the highest peak was on the 10<sup>th</sup> day but the possibility could still increase until an unknown day. *Spirulina* sp. culture will stay alive as long as the nutrients in the growth media are still available. Decreased density of *Spirulina* sp. in treatment A on the 6<sup>th</sup> day until the 9<sup>th</sup> day was caused by aeration on the A3 culture bottle died, thereby reducing the average value of density.

**Table 1.** Growth Rate of *Spirulina* sp. in Control and 3 Treatment.

Sample	Day										$\bar{x}$
	1	2	3	4	5	6	7	8	9	10	
K	0.02302	0.01650	0.01157	0.00943	0.00618	0.00356	-0.00043	-0.00214	-0.01058	-0.00941	0.00477
A	0.02501	0.02804	0.01045	0.01015	0.00566	-0.00042	-0.00049	-0.00017	0.00060	0.01183	0.00906
B	0.02904	0.00416	0.01852	0.01342	0.00382	0.00133	0.00192	-0.00510	-0.01156	-0.00736	0.00482
C	0.02518	0.02629	0.01328	0.00052	0.00727	0.00218	0.00343	0.00227	0.00422	0.00188	0.00865

Note: A = medium with 0.36 g/500 ml urea; B = medium with 0.043 g/500 ml NaHCO<sub>3</sub>; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml NaHCO<sub>3</sub>; K = control medium without addition of urea and NaHCO<sub>3</sub>.



**Figure 2.** Growth pattern of *Spirulina* sp. based on average density of control and treated medium. A = medium with 0.36 g/500 ml urea ; B = medium with 0.043 g/500 ml NaHCO<sub>3</sub>; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml NaHCO<sub>3</sub>; K = control medium without addition of urea and NaHCO<sub>3</sub>.

The data density of *Spirulina* sp. above showed that each treatment has a different standard deviation. The standard deviation on treatment A, B, C and control treatment (K) in a row were 0.161, 0.115, 0.168, and 0.114. The observations showed that treatments A and C were treated with the highest OD values compared to treatments K (control) and B. Treatment A and C contained the addition of urea nutrients as a source of N (nitrogen). The nitrogen content in urea added in treatments A and C has been shown to increase the growth of *Spirulina* sp. when compared with the control treatment (K). According to Ambarwati *et al.* (2018), the addition of N elements in the cultivation of microalgae with the right amount can optimally increase the population of microalgae. Rauf *et al.* (in Ambarwati *et al.*, 2018) state that in the growth of microalgae, N element plays a role in stimulating vegetative growth and increasing the number of microalgae cells. The increase in growth seen in the growth curve in treatments A and C proved an increase in the growth of *Spirulina* sp. until the last day of cultivation.

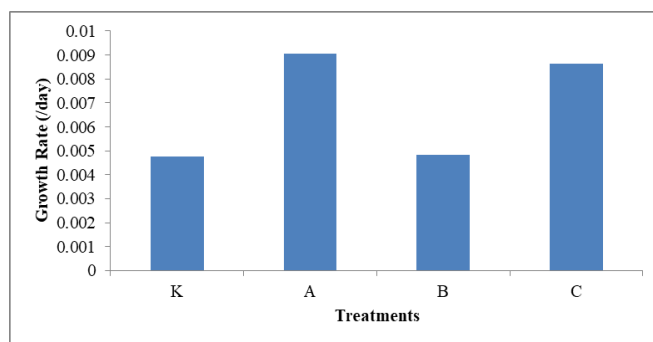
Based on the results of normality and homogeneity tests, data on *Spirulina* sp. are normal

and homogeneous ( $p > 0.05$ ). However, the results of the analysis using the ANOVA test showed a significant value is 0.132 ( $p > 0.05$ ), so that further tests could not be carried out. These results indicate that the addition of nutrients in the form of urea and NaHCO<sub>3</sub> does not affect the density of *Spirulina* sp.

### Growth Rate of *Spirulina* sp.

In this study, calculation of the growth rate (growth rate/day) is used to determine the rate of growth of *Spirulina* sp. per day. The results of the calculation of the growth rate are presented in Table 1.

Based on the *Spirulina* sp. growth rate data above showed that each treatment has a different standard deviation as same as the density. The standard deviation on treatment A, B, C, and control treatment (K) in a row that are 0.01041, 0.01241, 0.00971, and 0.01082. The average growth rates of *Spirulina* sp. in each treatment are presented in Figure 3.



**Figure 3.** Comparison of the average growth rate of *Spirulina* sp. in control and treatments medium. A = medium with 0.36 g/500 ml urea; B = medium with 0.043 g/500 ml NaHCO<sub>3</sub>; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml NaHCO<sub>3</sub>; K = control medium without addition of urea and NaHCO<sub>3</sub>.

Observation of growth rate (k) *Spirulina* sp. in each treatment has different results. The highest average growth rate of *Spirulina* sp. was in treatment A which was 0.00906/day followed by treatment C which was 0.00865/day, while the lowest average

growth rate of *Spirulina* sp. was in the control treatment (K) which was 0.00477/day followed by treatment B which was 0.00482/day. Based on the results of normality and homogeneity, growth rate data of *Spirulina* sp. normal and homogeneous ( $p > 0.05$ ). However, the results of the ANOVA test showed a significant value is 0.707 ( $p > 0.05$ ), so that further tests could not be carried out. Based on the ANOVA test results, the addition of nutrients such as urea and  $\text{NaHCO}_3$  did not affect the growth rate of *Spirulina* sp.

### Growth Phase of *Spirulina* sp.

Based on the growth pattern curve of *Spirulina* sp. in Figure 2, it can be analyzed the growth phases of *Spirulina* sp. which include:

#### Lag Phase / Induction Phase

According to Suyono and Winarto (2006), the lag phase is also referred to as the resting phase. On the growth pattern curve *Spirulina* sp. in Figure 3.2 the lag phase of each treatment occurred on day 0 and the 1<sup>st</sup> day. The density of *Spirulina* sp. in the control treatment (K) on day 0 was 0.147 cells/ml, treatment A was 0.142 cells/ml, treatment B was 0.143 cells/ml, and treatment C was 0.153 cells/ml. The density of each treatment which increased on the 1<sup>st</sup> day of cultivation where the control treatment (K) reached 0.219 cells/ml, treatment A reached 0.218 cells/ml, treatment B reached 0.235 cells/ml and treatment C reached 0.235 cells/ml. Differences density in each treatments are control treatment (K) of 0.072 cells / ml, treatment A of 0.076 cells/ml, treatment B of 0.092 cells/ml, and treatment C of 0.082 cells/ml. The increase in density on day 0 to the 1<sup>st</sup> day shows that the culture of *Spirulina* sp. has adapted to the media and its cultural environment. As according to Suyono and Winarto (2006), the lag time depends on cell viability, which is the possibility of cells to be able to live adjusting their environmental conditions. At a young inoculant (exponential phase), *Spirulina* sp. the possibility is still viable and adapts more quickly to the environment, whereas at the age of the older inoculants (stationary phase), the lag phase will last longer. *Spirulina* sp. culture which is used as an inoculant in this study is an inoculant with the age of 10 days which is likely to be in an exponential phase so that the lag phase only lasts for 1 day.

#### Log Phase

In this phase, the number of *Spirulina* sp. has increased rapidly. According to Suyono and Winarto (2006), this phase proves that microalgae cells have successfully adapted to the new growth media and utilize the nutrients contained in the media. Based on the growth pattern of *Spirulina* sp. in figure 3.2,

the exponential phase of control treatment (K) lasts from the 1<sup>st</sup> day to the 5<sup>th</sup> day, treatment A takes place from the 1<sup>st</sup> day to the 5<sup>th</sup> day, treatment B takes place from the 1<sup>st</sup> to the 6<sup>th</sup> day, and treatment C lasts from the 1<sup>st</sup> day to the unknown day if cultivation time is continued.

#### Stationary Phase

In this phase, the growth of *Spirulina* sp. stationary or permanent. This shows that the growth rate of *Spirulina* sp. the same as the rate of death. Based on the growth pattern of *Spirulina* sp. in figure 3.2, the stationary phase of the control treatment (K) lasts from the 6<sup>th</sup> day to the 7<sup>th</sup> day, treatment A takes place from the 5<sup>th</sup> to the 9<sup>th</sup> day, treatment B takes place on the 7<sup>th</sup> day, and C treatment is thought to have not yet reached the stationary phase.

#### Death Phase

In this phase, the decreased growth of *Spirulina* sp. higher than the stationary phase, so the growth pattern tends to decrease. Based on the growth pattern of *Spirulina* sp. in figure 3.2, the death phase in the control treatment (K) lasted from the 8<sup>th</sup> day to the 10<sup>th</sup> day, in treatment A it was thought that it had not yet experienced a death phase because the cell density increased again on the 10<sup>th</sup> day, in treatment B the death phase lasted since the 8<sup>th</sup> day to the 10<sup>th</sup> day, and treatment C has not yet experienced a phase of death because cell density still continues to increase until the last day of cultivation.

### Maximum OD of *Spirulina* sp.

In this study, the density of *Spirulina* sp. measured every 24 hours for 10 days of cultivation. This is done to get the maximum density (maximum OD) of each treatment. In the control treatment (K), the density of *Spirulina* sp. was on the 6<sup>th</sup> day with a value of 0.492 cells/ml. In treatment A, the density of *Spirulina* sp. the highest was on the 10<sup>th</sup> day with a value of 0.672 cells/ml. In treatment B, the highest density of *Spirulina* sp. was on the 7<sup>th</sup> day with a value of 0.494 cells/ml, while in treatment C the highest density of *Spirulina* sp. was on the 10<sup>th</sup> day with a value of 0.674 cells/ml. The difference in density is due to each treatment. The growth media is different which causes the nutrient content in the growth media is also different. Thus, this is in accordance with the statement of Widianingsih (2008), that differences in cell density are caused by differences in nutrient content in growth media.

### CONCLUSION

The results indicated that addition of urea and  $\text{NaHCO}_3$  didn't affect to OD and *Spirulina* sp.

growth rate. OD illustrates population density meanwhile, the increasing OD indicates the growth of *Spirulina* sp. The growth of *Spirulina* sp. per unit of time is the growth rate. The highest growth rate was treatment A with addition 0.36 g/500 ml of urea without addition of NaHCO<sub>3</sub> supplements which had 0.00906/day, followed by treatment C with addition 0.36 g/500 ml of urea and 0.043 g/500 ml of NaHCO<sub>3</sub> supplements which had 0.00865/day. Treatment B with addition of 0.043 g/500 ml of NaHCO<sub>3</sub> without addition of urea and the control treatment showed a low growth rate, which were 0.00482/day and 0.00477/day. The maximum OD value obtained in treatment C with addition of 0.36 g/500 ml of urea and 0.043 g/500 ml of NaHCO<sub>3</sub> supplements was 0.674 cells/ml on the 10<sup>th</sup> day.

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