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Research Article

# Optimation of *Spirulina* sp. Growth in Walne Media with Variation of Urea and $NaHCO_3$ 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 $NaHCO_3$ 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 $NaHCO_3$, treatment B addition of 0.043 g/500 ml $NaHCO_3$ without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$, and control without addition of urea or $NaHCO_3$. The results indicated that addition of urea and $NaHCO_3$ 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 $NaHCO_3$ can be used as additional nutrients to fulfill the survival of *Spirulina* sp. Urea is a source of nitrogen (N) while $NaHCO_3$ is a source of carbon (C). Urea and $NaHCO_3$ 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 $NaHCO_3$ 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 $NaHCO_3$ supplies on OD and the growth rate of *Spirulina* sp., to find out the value of *Spirulina* sp.growth rate with urea and $NaHCO_3$ 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 $NaHCO_3$, treatment B addition of 0.043 g/500 ml $NaHCO_3$ without addition of urea, treatment C addition of 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$, and control without addition of urea or $NaHCO_3$. 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 $B_{12}$ 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

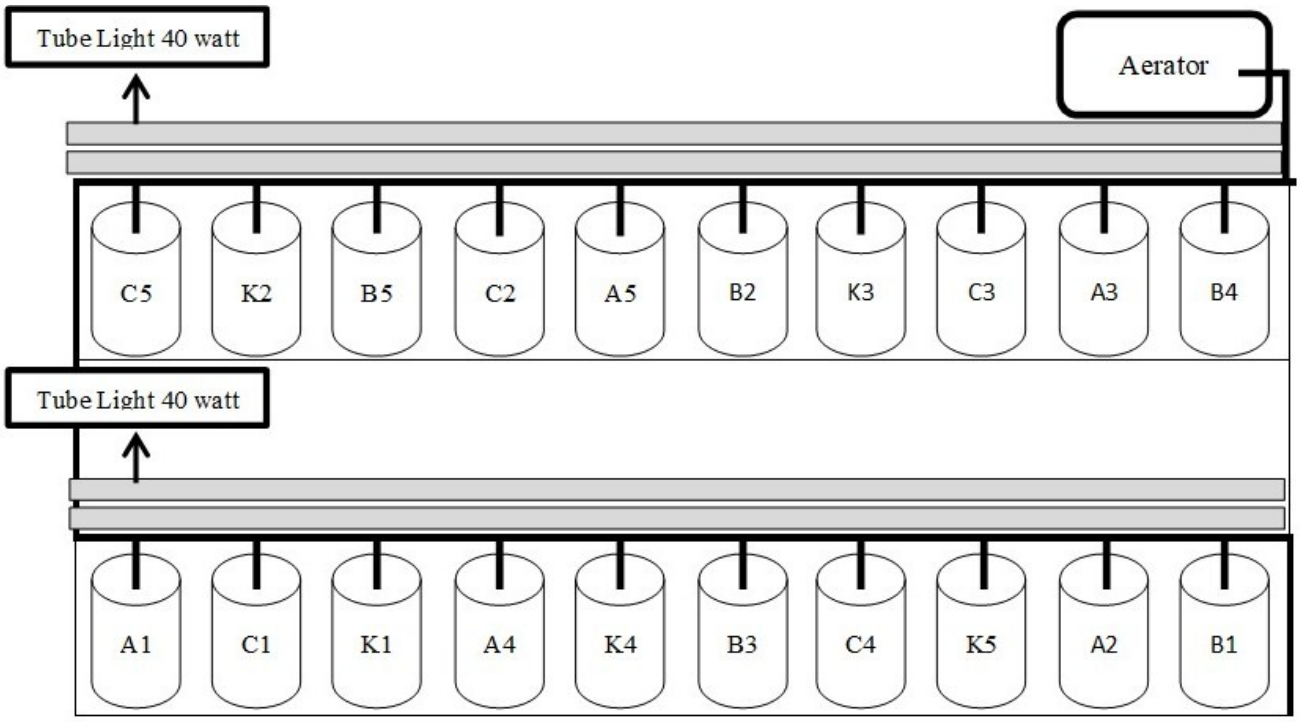


**Figure 1.** Design of cultivation tools.

quantitatively using the ANOVA test on IBM SPSS Statistics version 20. In this study, the X variable is the addition of urea and $NaHCO_3$, 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 0th day of cultivation to the 10th day. Based on Figure 2, it's shown that the highest density value on treatment K (control) was on the 6th day which was 0.492 cells/ml, treatment A on the 10th day which was 0.672 cells/ml, treatment B on the 7th day which was 0.494 cells/ml, and treatment C on the 10th 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 1st 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 6th day, but decreased from the 7th day to the 10th day. The density of *Spirulina* sp. treatment A continued to increase from the 1st day of cultivation to the 5th day, then down to the 9th day, and increased again on the 10th day. The density of *Spirulina* sp. treatment B continued to increase since the 1st day of cultivation and experienced a peak on the 7th day, then decreased from the 8th to the 10th day, while the density of *Spirulina* sp. treatment C has increased since the 1st day of cultivation and continues to increase until the 10th day. In treatments A and C, the highest peak was on the 10th 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 6th day until the 9th 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 | | | | | | | | | | ẋ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 $NaHCO_3$; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$; K = control medium without addition of urea and $NaHCO_3$.

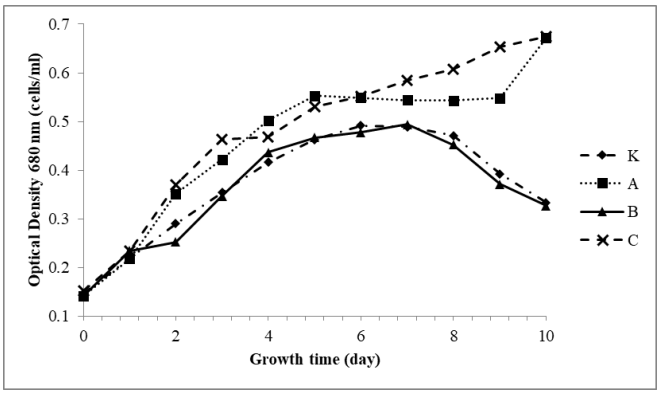


**Figure 2.** Growth pattern of *Spirulina* sp. based on average density of control and treated medium. A = medium with 0.36 g/500 ml urea ; B = medium with 0.043 g/500 ml $NaHCO_3$; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$; K = control medium without addition of urea and $NaHCO_3$.

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 $NaHCO_3$ 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.

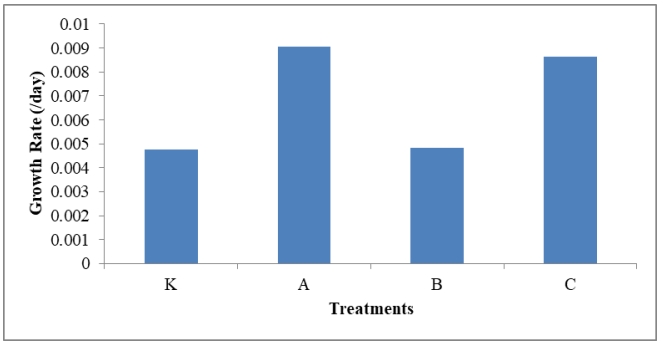


**Figure 3.** Comparison of the average growth rate of *Spirulina* sp. in control and treatments medium. A = medium with 0.36 g/500 ml urea; B = medium with 0.043 g/500 ml $NaHCO_3$; C = medium with 0.36 g/500 ml urea and 0.043 g/500 ml $NaHCO_3$; K = control medium without addition of urea and $NaHCO_3$.

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 $NaHCO_3$ 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 1st 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 whichincreased on the 1st 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 1st 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 1st day to the 5th day, treatment A takes place from the 1st day to the 5th day, treatment B takes place from the 1st to the 6th day, and treatment C lasts from the 1st 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 6th day to the 7th day, treatment A takes place from the 5th to the 9th day, treatment B takes place on the 7th 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 8th day to the 10th day, in treatment A it was thought that it had not yet experienced a death phase because the cell density increased again on the 10th day, in treatment B the death phase lasted since the 8th day to the 10th 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 6th day with a value of 0.492 cells/ml. In treatment A, the density of *Spirulina* sp. the highest was on the 10th day with a value of 0.672 cells/ml. In treatment B, the highest density of *Spirulina* sp. was on the 7th day with a value of 0.494 cells/ml, while in treatment C the highest density of *Spirulina* sp. was on the 10th 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 NaHCO3 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 $NaHCO_3$ 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 $NaHCO_3$ supplements which had 0.00865/day. Treatment B with addition of 0.043 g/500 ml of $NaHCO_3$ 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 $NaHCO_3$ supplements was 0.674 cells/ml on the $10^{th}$ day.

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## REFERENCES

Ambarwati, Diyah Putri, Ervia Yudiati, Endang Supriyantini, and Lilik Maslukah, 2018, 'Pola Pertumbuhan, Biomassa dan Kandungan Protein Kasar pada Kultur Mikroalga *Skeletonema costatum* Skala Massal dengan Konsentrasi Kalium Nitrat ($KNO_3$) yang Berbeda', *Buletin Oseanografi Marina*, 2 (7), pp 75 -80.

Christwardana, Marcellinus, Muhamad Maulana Azimatun Nur, and H. Hadiyanto, 2012, '*Spirulina platensis*: Potensinya Sebagai Bahan Pangan Fungsional', *Jurnal Aplikasi Teknologi Pangan*, 1 (2) pp 1-4.

Dewi, Sri Suminar, 2016, '*Teknologi Membran dalam Produksi Bioetanol*', Institut Teknologi Bandung, pp 1-6.

Hadiyanto, and Maulana Azim, 2012, '*Mikroalga: Sumber Pangan dan Energi Masa Depan*', UPT UNDIP Press, Semarang, pp 1-18.

Juneja, Ankita, Ruben Michael Ceballos, and Ganti S. Murthy, 2013, 'Effect of Environmental Factors and Nutrien Availability on the Biochemical Composition of Algae for Biofuels Production', *Journal Energies*, (6) pp 4607-4638.

Kawaroe, Mujizat, Tri Prartono, and Ganjar Saefurahman, 2015, 'Kepadatan dan Laju Pertumbuhan Spesifik *Nannochloropsis* sp. pada Kultivasi Heterotropik Menggunakan Media Hidrolisat Singkong', *Jurnal Omni-Akuatika*, 11 (2) pp 15-19.

Melanie, Susiana and Diini Fithriani, 2015, 'Rendemen Minyak dari Mikroalga *Spirulina* sp. dan *Chlorella* sp. dengan Teknik Pemecahan Dinding Sel', *Jurnal Widyariset*, 1 (1) pp 61-70.

Muyassaroh, Rini Kartika Dewi, and Dwiana Anggorowati, 2018, 'Kultivasi Mikroalga *Spirulina platensis* dengan Variasi Pencahayaan Menggunakan Lampu TL dan Matahari', *Prosiding Seminar Nasional Aplikasi Sains & Teknologi (SNAST),* Pp 381-386.

Prayitno, Joko, 2016, 'Pola Pertumbuhan dan Pemanenan Biomassa dalam Fotobioreaktor Mikroalga untuk Penangkapan Karbon', *Jurnal Teknologi Lingkungan*, 1 (17) pp 45-52.

Sari, Fitria Yuli Anggita, I Made Aditya Suryajaya, and Hadiyanto, 2012, 'Kultivasi Mikroalga *Spirulina platensis* dalam Media POME dengan Variasi Konsentrasi POME dan Komposisi Jumlah Nutrien', *Jurnal Teknologi Kimia dan Industri*, 1 (1) pp 487-494.

Seftian, Deky, Ferdinand Antonius, and M. Faizal, 2012, 'Pembuatan Etanol dari Kulit Pisang Menggunakan Metode Hidrolisis Enzimatik dan Fermentasi', *Jurnal Teknik Kimia*, 1 (18) pp 10-16.

Suyono, Eko Agus, and Winarto Haryadi, 2006, 'Optimasi Media untuk Produksi Biomassa Mikroalga *Chaestoceros* sp dan *Skeletonema* sp Isolat Jepara dan Analisis Kandungan Asam Lemaknya', *Jurnal Sains dan Teknologi*, Pp 6-8.

Syaichurozzi, Iqbal, and Jayanudin, 2016, 'Kultivasi *Spirulina platensis* pada Media Bernutrisi Limbah Cair Tahu dan Sintetik', *Jurnal Bahan Alam Terbarukan*, 5 (2) pp 68-73.

Widianingsih, Ali Ridho, Retno Hartati, and Harmoko, 2008, 'Kandungan Nutrisi *Spirulina platensis* yang Dikultur pada Media yang Berbeda', *Jurnal Ilmu Kelautan*, 3 (13) pp 167-170.