DOI: https://doi.org/10.36722/exc.v1i1.2349

The Impact of Incorporating Phosphor Into Liquid Organic Fertilizer Media on The Production of Pigment and Protein in *Spirulina* sp.

Wendy Almira Kintoko[1], Risa Swandari Wijihastuti[1], Nita Noriko[1]

[1]*Department of Biotechnology, Faculty of Science and Technology, University of Al-Azhar Indonesia, Sisingamangaraja, South Jakarta, 12110, Indonesia*
Correspondence Email: risa.swandari@uai.ac.id

ABSTRACT

Spirulina sp, a type of blue-green microalgae, possesses various beneficial properties such as antioxidants, probiotics, proteins, pigments, and carbohydrates, enabling it to generate a wide range of useful products. Previous studies using commercial fertilizers as the growth medium, have not achieved optimal pigment and protein production. Therefore, this study aims to investigate the impact of combining liquid organic fertilizer derived from Canna indica waste with TSP (Triple Super Phosphate) fertilizer on changes in pigment and protein production in Spirulina sp. The study was conducted for 16 days using a completely randomized design, with four replicates for each of the six treatments, along with a control. The results demonstrated a prolonged logarithmic growth phase, with the highest dry biomass weight observed on the 16th day in the treatment 1A (1,585 gr/L). Treatment 1A exhibited the highest levels of allophycocyanin and phycoerythrin pigments (9,861 mg/L and 4,319 mg/L, respectively), while treatment 1C yielded the highest protein content (0.33%). Statistical analysis on day 12, with a 95% confidence level, revealed significant differences in pigment production between various treatment groups. The study suggests that a phosphor concentration of 20 ppm is optimal for cultivating Spirulina sp.

Keywords*: Fertilizer, Phosphor, Phycobiliprotein, Pigment, Spirulina sp*

1. INTRODUCTION

Microalgae is a photosynthetic organism with high growth rate and can produce a variety of useful products, one of which is Spirulina sp. Spirulina sp. is a blue-green microalgae that is classified into Cyanobacteria and is a multicellular organism [1], spirulina sp. lives in an optimal environment with a pH ranging from 8.5-10.5, a temperature of 25-30°C, and a light intensity of 1500-3500 lux [2].

Spirulina sp. is widely used for consumption in the form of supplements, a food mixture, and as a disease treatment therapy because it contains antioxidants, phytonutrients, and nutraceuticals [3][4], spirulina sp. become one of the food sources in Mexico and Africa. National Aeronautics and Space Administration (NASA) has also selected Spirulina sp. as a food source in the future [5].

Microalgae growth is influenced by two factors, namely supporting factors (non-resource) and resource factors (resource). Supporting factors consist of environmental factors that affect metabolic processes in microalgae cells, such as pH and temperature. Meanwhile, resource factors consist of resources that are directly used by microalgae cells for their growth, such as CO2, sunlight, and nutrients [6], one of the very influential nutrients is phosphorus which is influential in the formation of chlorophyll-a, protein, and microalgae carbohydrates. Without the presence of phosphorus, microalgae cannot grow and develop optimally because phosphorus as a macronutrient plays a role in the preparation of protein compounds [7].

However, the culture of Spirulina sp. usually cultured using relatively expensive commercial fertilizers, such as Pro Analysis fertilizer [8], therefore, this study used liquid organic

fertilizer from Canna discolor waste as an alternative media mixture. Spirulina sp. in several studies it has been successfully grown on liquid fertilizer media which is generally made from vegetable waste and other agricultural products, but unfortunately it has not been able to optimize the protein and pigment production of Spirulina sp. Therefore, in this study, the addition of phosphorus content in the form of TSP (Triple Super Phosphate) fertilizer was carried out as a source of phosphate in the growing medium for Spirulina sp. in the hope that it will increase the production of proteins and pigments.

Protein in Spirulina sp. is a complex compound containing 20 different types of amino acids bonded together. Several amino acids in Spirulina sp. are methionine (1.3-2.75%), cystine (0.5-0.7%), tryptophan (1-1.95%), and lysine (2.6-4.63%). High levels of amino acids are good for health because they are one of the building blocks of protein [9][10][11]

There are two types of nutrients, namely macro nutrients such as N, P, K, S, Si and C, and micro nutrients such as Fe, Mo, Cu, Ca, Mn, Zn, and Co. The medium used by an organism to grow and develop is called a growing medium. Nutrients or fertilizers are important elements in Spirulina sp. media. to maintain the quantity, quality, and stability of cell production, such as macronutrients and micronutrients [12].

Growth factors include internal factors and external factors. Internal factors are factors from within the organism's body that can affect the growth of the organism in terms of genes and hormones, while external factors are factors from outside the organism which include external elements, such as nutrients, planting media, temperature, humidity, light intensity, and water [13][14].

Phosphorus functions as a stabilizer of cell membranes in energy metabolism, and regulation of microalgae metabolism. Phosphorus is an important constituent of ATP (Adenosine Tri Phosphate) which serves as the basic material for the formation of nucleic acids, enzymes, and plays a role in the biosynthesis of nucleic acids and the transfer of energy in the process of photosynthesis [15], spirulina sp. Biomass increases with the addition of phosphate concentration, but the addition of concentration must be in accordance with the culture scale so as not to cause turbidity which leads to disruption of the photoautotrophic process and inhibits the growth of Spirulina sp.[7], phosphorus is very necessary as the transfer of energy from the outside into the organism's cells, so that phosphorus is one of the main elements needed for microalgae growth and increases microalgae biomass [16][17], lack of concentration of phosphorus which acts as a constituent of protein compounds can cause a decrease in protein content and is followed by degradation of cell components related to protein synthesis, including chlorophyll-a and phycobiliproteins [7].

2. METHOD

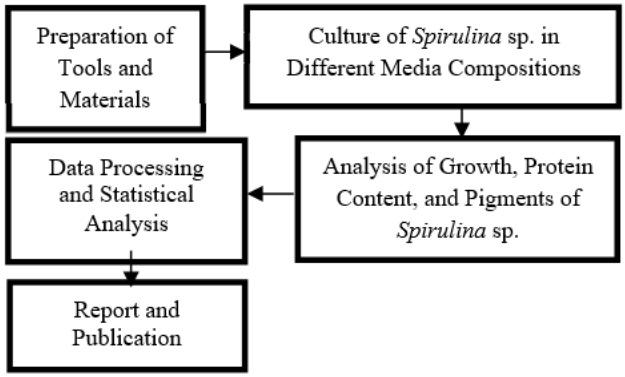
A. Design, Place, and Time

This research was conducted from September to December 2022. Inoculant Spirulina sp. was taken from the Surfactant and Bioenergy Research Center of the Bogor Agricultural Institute (SBRC IPB). While all activities are carried out in the Chemistry Laboratory of the Biology Department, University of Al Azhar Indonesia.

B. Tools and Materials

Some of the tools used in this study are UV Vis Spectrophotometer, Vortex, oven, aerator, LED lamp, aeration hose, micropipette, centrifugation tube, water bath, desiccator, plastic bottle, analytical balance, glassware, aluminum foil, and Sedgewick rafter. The materials used include Whatman GF/C glass fiber filter paper (47 mm), TSP fertilizer, distilled water, vitamins, liquid nitrogen, biuret reagent, folin phenol reagent, BSA (Bovine Serum Albumin), and NaNO3.

C. Research Stages



D. Preparation and Cultivation of Spirulina sp.

Spirulina sp. culture seeds. amplified to have a cell density of 104 units/mL and counted with the Sedgewick Rafter chamber. The combination of POC and TSP fertilizer concentrations is

presented in table 1. The final volume of culture to be made in the Spirulina sp. is as much as 1000mL, where the culture will be grown in light at ± 2500 lux, room temperature ranging from 19-23°C, 24 hours of aeration, and grown up to 16 days. There were 4 repetitions for 6 treatments and 1 control.

Table 1. Growth Media Fertilizer Media

Treatment	*Canna discolor* Fertilizer Concentration (ppm)	TSP Fertilizer Addition Concentration (ppm)
Control	18.000	-
1A	20.000	20
1B	20.000	30
1C	20.000	40
2A	22.000	20
2B	22.000	30
2C	22.000	40

E. Growth Analysis

Spirulina sp. growth observation were performed every day for 16 days. The growth rate was calculated in the logarithmic phase and weight of dry biomass and growth rate of Spirulina sp. counted at the end of the culture period. The filter paper was heated in an oven at 100°C for 1 hour. Then, the filter is weighed and put into the desiccator. Then, as much as 10 mL of Spirulina sp. filtered into a filter, heated in an oven for 1 hour, put in a desiccator for 24 hours, and weighed. Spirulina sp. dry biomass weight calculated by this formula:

Meanwhile, the growth rate refers to Borowitzka & Beardall [8] and calculated by the following formula:

$$K' = \frac{Ln\left(\frac{N_{t2}}{N_{t1}}\right)}{t2-t1} \quad \mu = \frac{K'}{Ln\,2}$$

Notes:

K' : Cleavage time (day-1)
μ : Specific growth rate (g/L/day)
Nt1 : Dry weight at time 1 (g/mL)
Nt2 : Dry weight at time 2 (g/mL)
t1 : Time 1
t2 : Time 2

F. Protein Protein Content

Analysis of protein content was carried out by making samples using the Lowry method. First, the extracted Spirulina sp. was prepared in a 10 mL centrifugation tube and crushed with liquid nitrogen. Then, mix the extracted Spirulina sp. with 1 mL, 1 mL, 3 mL, and 5 mL of biuret reagent gradually. Then wait for 20 minutes and add 0.5 mL of folin phenol reagent. Next, stir the sample using a Vortex and leave it for 10 minutes. Finally, remove the supernatant by filter and read the absorbance at 660 nm. Determine the protein content by the standard curve of the standard solution as follows:

Table 2. Standard Solution Composition

Protein	0	50	100	150	200	250	300	350
BSA (mL)	0.00	0.02	0.04	0.06	0.08	0.10	0.12	0.14
dH_2O (mL)	0.14	0.12	0.10	0.08	0.06	0.04	0.02	0.00

G. Pigmen Content Analysis

Pigment content analysis was carried out according to Borowitzk and Moheimani [13]. Phycobiliprotein pigments are obtained from an extraction process, namely pounding with liquid nitrogen, then K-phosphate buffer (KH2PO4 and K2HPO4) or NaNO3, then refrigerate for 24 hours. After that, the supernatant in the extract was filtered and mixed with buffer. Then the absorbance was measured with a spectrophotometer at a wavelength of 650nm, 620nm and 565nm. The phycobiliprotein concentration was calculated using the following formula:

3. RESULT AND DISCUSSIONS

A. Growth Analysis

The research began with the preparation of Liquid Organic Fertilizer (POC) and Spirulina sp. inoculant culture. Fertilizer that has been successfully fermented for 14 days has a characteristic smell of fermentation, is yellowish brown in color, and is watery in nature. The density of the number of inoculant culture cells that are uniform (Figure 1) will help to accurately determine the growth of microalgae [18].

Figure 1. Spirulina sp. Inoculant Culture

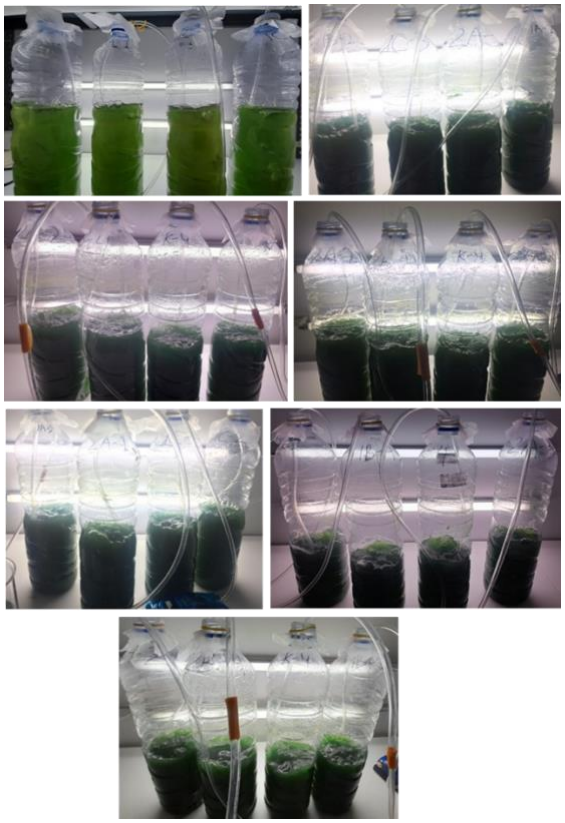


Figure 2. Culture Treatment of Spirulina sp. (RAL) Day 0 to Day 16

Microalgae growth is divided into a lag phase, logarithmic phase, growth rate reduction phase, stationary phase, and death phase [1], growth chart of Spirulina sp, shows that the lag phase of all treatments and controls is not clearly visible due to the fast lag phase which turns into a logarithmic phase in the first two days of culture. The logarithmic phase was indicated by the increase in the curve until day 16. This indicated that the culture of Spirulina sp. coupled with TSP able to adapt well. The lag phase curve that is not clearly visible does not mean that the lag phase does not occur, but because the lag phase occurs very quickly which causes the growth phase to be difficult to describe by the growth curve [19].

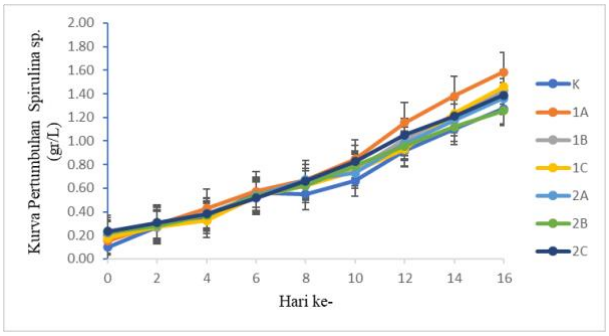


Figure 3. Spirulina sp. Growth

Until the 16th day Spirulina sp. are still in the logarithmic phase, a phase where cells are actively dividing and the growth rate increases rapidly, which increases the population density. It was at this time that the formation of proteins and the constituent components of plasma cells increased due to the photosynthetic activity of Spirulina sp [16], the high nutrient content at the beginning of the culture period is utilized by Spirulina sp. for cell reproduction and growth, it describes a long logarithmic phase [20], in previous studies (unpublished data), Spirulina sp. was grown with POC had entered the stationary phase until the death phase on the 16th day.

After logarithmic phase, microalgae will enter a stationary phase or referred to as the population peak, where growth occurs slowly because the growth and death race in cells are in balance. This happens because the number of population increases but nutrients are not added, and it can also occur due to self-shading or the formation of cell shadows along with increasing cell density which causes reduced light absorption and lowers growth rate [21], the last phase of life is the death phase, which is when there is a decrease in the number of cells in all treatments after the culture reaches its peak because the death rate is higher than the cell growth rate and it can be said that the cells are no longer dividing, so the population density will decrease [22], however, in this study, the culture of Spirulina sp. has not yet been seen

entering the stationary phase and the death phase.

The results of ANOVA test with a degree of confidence of 95% showed a significant difference in the increase in biomass weight between the treatment cultures of group 1A and the control in the table 3. The control culture experienced the highest growth rate (0.23g g/L) but the dry biomass was lower, namely 1.27 g/L on the 16th day. This can happen because there is no TSP concentration which encourages the addition of Spirulina sp biomass, but provides space for light to enter and increase the growth rate.

Table 3. Dry Biomass Weight, Growth Rate, Protein, and Pigments Content of Spirulina sp.

Perlakuan	Berat Biomassa Kering (g/L)	Laju Pertumbuhan (g/L/hari)	PC (mg/L)	PE (mg/L)	APC (mg/L)	Protein (%)
2B	1.25 ± 0.180	0.154 ± 0.02[a]	4.031	1.919	4.898	0.30
2C	1.38 ± 0.236	0.160 ± 0.03[a]	3.111	1.773	4.992	0.27
2A	1.36 ± 0.235	0.164 ± 0.009[a]	3.949	2.212	5.594	0.25
1B	1.42 ± 0.254	0.183 ± 0.01[a]	2.398	1.506	3.850	0.28
1C	1.45 ± 0.176	0.190 ± 0.01[a]	3.712	2.302	5.417	0.33
1A	1.58 ± 0.238	0.211 ± 0.02[b]	6.250	4.319	9.861	0.29
Kontrol	1.27 ± 0.193	0.232 ± 0.01[a]	7.288	4.015	9.417	0.30

*The same letter indicates no significant difference.
*PC: Fikosianin; PE: Fikoeritrin; APC: Allofikosianin.

The highest treatment culture yield was in the control group with a rate of 0.232 g/L/day, but based on statistical tests, treatment group 1A had a significant difference from the control and other treatments. This happened because of the compatibility between Spirulina sp. of nutrients in growth media. The growth rate of Spirulina sp. influenced by the source of nutrition and the environment [23].

Treatment cultures with a POC media concentration of 20,000 ppm had higher dry biomass than treatment cultures with a POC media concentration of 22,000 ppm. The low specific growth rate of groups 2A, 2B, and 2C indicates the possibility of saturation of POC elements in the culture because the higher the concentration of POC given, the higher the concentration of cells produced and causes self-shading so that the culture of Spirulina sp. with a POC media concentration of 22,000 ppm showed lower growth performance [1].

Microalgae utilize phosphate in the form of orthophosphate (HPO4-), because it only requires a certain amount of phosphate. Phosphorus is only needed in certain amounts for the growth of microalgae, because excessive phosphorus content will inhibit the process of assimilation or adaptation of phosphorus compounds to the growth of microalgae. The function of phosphate for the growth of Spirulina sp. is the site of cell division, protein constituent, and part of the cell nucleus. Phosphorus deficiency can interfere with formation of ATP and cause limited cell growth. Lack of phosphorus in the control group causes a decrease in biomass weight, because phosphorus acts as a cell membrane stabilizer and regulates microalgae metabolism. Inhibited synthesis of proteins and carbohydrates can reduce the pigment content [24].

All samples underwent a long logarithmic phase because cells continued to divide rapidly and constantly following a logarithmic curve. Factors that affect the logarithmic phase are light, nutrients, the surrounding environment, and water quality [25], the increase in dry weight indicated that nutrients and light were the main factors in the growth of Spirulina sp. through the process of photosynthesis [22], provision of continuous light can also affect changes in cell concentration in culture, because light plays a role in photosynthesis which affects the biochemical and genetic makeup of cells [20].

Insufficient light intensity will reduce the growth rate of Spirulina sp [25][26], also showed success in growing Spirulina sp. on POC media without added phosphate. The results show that this fertilizer can only increase the growth rate and biomass production of Spirulina sp. as much as 0.68 g/L until the 6th day and after that some cultures entered the stationary phase.

B. Pigment Content Analysis

Phycobiliproteins can dissolve in water and are found in the algae Cyanophyceae [27].

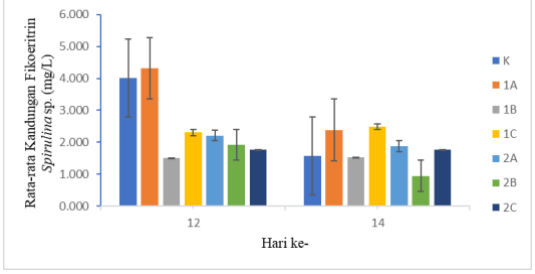


Figure 4. Graph of Phycoerythrin Pigment Content in *Spirulina* sp.

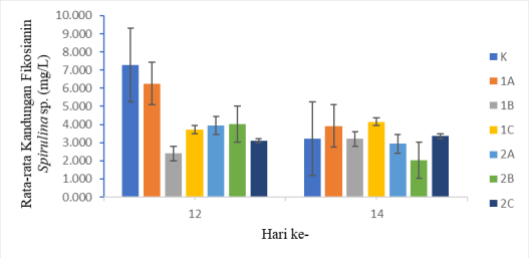


Figure 5. Graph of Phycoerythrin Pigment Content in *Spirulina* sp.

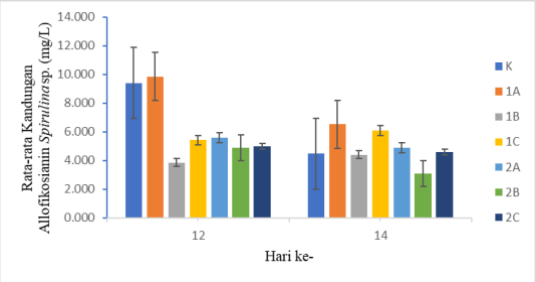


Figure 6. Graph of Allophycocyanin Pigment Content in *Spirulina* sp.

The highest content of phycoerythrin and allophycocyanin pigments were 4.319 mg/L and 9.861 mg/L respectively by 1A on the 12th day. Meanwhile, the highest content of phycocyanin pigment was 7.288 mg/L by the control group on the 12th day. The graph of phycoerythrin content shows that on day 12 treatment group 1A had a significant difference from groups 1B and 2C. The phycocyanin content graph shows that TSP fertilizer did not have a significant effect on the phycocyanin pigment content because the highest phycocyanin pigment content on day 12 was in the control group at 7.288 mg/L. The statistical test results showed that on the 12th day there was a significant difference between the control group and the treatment groups 1B, 1C, and 2C. Whereas in the allophycocyanin pigment content variable on the 12th day, there was a significant difference between the 1A and 1B treatment groups.

The productivity of phycobiliproteins is influenced by microalgae strains and cultivation parameters such as culture media, light, aeration, and pH.. Phycobiliproteins are formed by transcriptional, translational, and post-translational processes which include the synthesis of amino acids, proteins, and phycobilins, as well as the ligation of phycobilins which are synthesized into apoproteins during the post-translational phase. [28], differences in the content of phycobiliprotein pigments can be caused by pigment instability which is affected by light, oxygen, temperature, and pH [29], turbidity in the culture of Spirulina sp. which are cultivated with TSP and POC media allow light to be absorbed so that the production is not optimal. Thus, turbidity or increased water turbidity can cause the content of phycobiliprotein pigments to decrease [29], the phycobiliprotein content is also affected by light intensity, so the phycobiliprotein results obtained from this study are higher than the results of research by Aryono [30], who used a 2000 lux LED lamp on the growth of Spirulina sp.

The highest production of phycocyanin pigments is in the late logarithmic phase, that is before entering the stationary phase because phycocyanin as a secondary metabolite is produced during the differentiation stage into specialized cells. The yield of phycoerythrin is the lowest because phycoerythrin works as a complementary pigment to help chlorophyll-a absorb light during photosynthesis. The light absorbed by phycoerythrin is transferred to phycocyanin, allophycocyanin, and to chlorophyll. Meanwhile, according to the literature, pigment content can decrease with increasing turbidity such as increasing biomass weight due to the large amount of TSP and POC content in treatments 2A, 2B, and 2C. So the lower phycoerythrin content can occur due to increased production of phycocyanin and allophycocyanin [31].

Phycoerythrin, phycocyanin, and allophycocyanin are active components that are not resistant to heat, so the pigments will be damaged or evaporate if extracted at high temperatures. Changes in cultivation conditions such as light intensity and quality, temperature, and media concentrations such as carbon and nitrogen can affect pigment production in all strains [32], the longer the extraction time, the longer the contact time between the sample and the solvent which causes more and more compounds to be extracted. The amount of substance extracted will increase the temperature, but too high a temperature can damage the material being processed [33].

C. Analysis of Protein Content

The ANOVA test analysis results with a significance value of 5% showed that the concentration of TSP did not significantly affect the protein content of Spirulina sp. Group 1A was significantly different from 2A and 2B, but group 1C had the highest average protein

content. The highest average protein content was produced by group 1C with a combination of 40 ppm TSP and 20,000 ppm POC on the 12th day when compared to the other treatment groups, namely 0.33% of the dry weight.

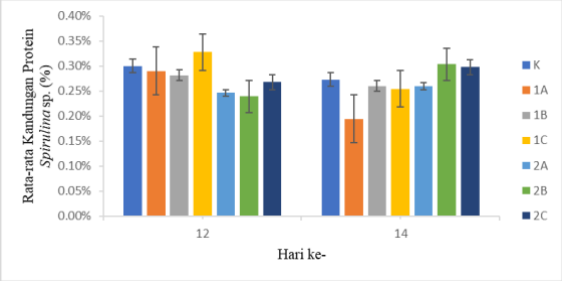


Figure 7. Average Protein Content of Spirulina sp.

Protein analysis was carried out using Biuret buffer to determine the peptide bonds that form protein molecules in the biomass. In alkaline conditions, the protein reacts with Cu2+ and forms a purple color complex whose absorbance is measured at a wavelength of 660nm. Nitrogen content in the form of NO3 and NO2 in the growth medium increases cell protein biomass, high nitrogen concentrations in the absence of phosphorus can increase protein content, but it is not as effective as if both are present [34].

The graph in figure 10 shows a decrease in protein levels in the 20,000 ppm POC treatment on the 14th day, this is suspected because the protein decomposes again because the food reserves resulting from photosynthesis do not meet the needs. If there is a shortage of food reserves, the protein will oxidize and hydrolyze into its constituent amino acids, which then decompose through glycolytic reactions and the Krebs cycle [1], the decrease in protein content is also suspected because harvest time is carried out at the end of the logarithmic phase so that it affects the process of amino acid biosynthesis. Harvesting at the end of this phase produces relatively the same amount of protein because in this phase there is rapid absorption of nutrients [35].

The protein content of Spirulina sp. Different conditions are influenced by environmental factors such as nutrients, light, temperature, and CO2. These factors affect photosynthesis and the productivity of cell biomass, as well as affect the shape and flow of cellular metabolic activities that have an impact on the dynamics of cell composition. Light is a source of energy in the process of photosynthesis. The amount of energy received is influenced by the quality, quantity and duration of irradiation. The cellular response of microalgae to low light intensity is to increase chlorophyll-a and other pigments as light harvesters. So treatment with a short light duration is believed to produce a higher protein [11]. The environmental conditions in this study were in accordance with the culture requirements of Spirulina sp. namely temperature 23°C, light 2500 lux, and continuous aeration [36]

The decrease in group 1C protein levels from 0.33% to 0.25% on day 14 was caused by a deficiency of nitrogen and phosphorus which could inhibit protein synthesis, because nitrogen is a macromineral that influences cell metabolic activity [37], which is an important element in protein formation. Nitrogen helps cells prepare amino acids and cell division, so it is important for the growth of Spirulina sp. According to Leksono [14], increasing the dose of liquid fertilizer can increase the nutrient content such as protein Spirulina sp. Therefore, to obtain Spirulina sp with high level of protein content require higher levels of nitrogen than phosphorus levels.

4. CONCLUSION

Growth media with a combination of POC from Canna discolor waste and TSP fertilizer with various concentrations had a significant effect on changes in the production of allophycocyanin pigments, phycoerythrin, and the dry biomass weight of Spirulina sp., but did not significantly affect changes in the production of phycocyanin pigments, protein, and the growth rate of Spirulina sp. The content of allophycocyanin pigments is the highest compared to phycocyanin or phycoerythrin pigments. POC concentrations of 18,000 – 20,000 ppm and TSP of 30 – 40 ppm can increase the protein and pigment biomass of Spirulina sp. Adding TSP to POC media did not significantly affect the protein content of Spirulina sp. because the best macronutrients to increase the protein content of Spirulina sp. is nitrogen. The addition of TSP to POC media had a significant effect on increasing the weight of Spirulina sp. dry biomass, but the lag phase was not clearly visible and there were several phases that were not visible within 16 days of the study.

REFERENCES

[1] Amanatin, D. & Nurhidayati, T., 2013. Pengaruh Kombinasi Konsentrasi Media Ekstrak Tauge (MET) dengan Pupuk Urea Terhadap Kadar Protein Spirulina sp. Jurnal Sains dan Seni Pomits, 2(2), pp. 182-185.

[2] Soni, R., Sudhakar, K. & Rana, R., 2019. Comparative Study on The Growth Performance of Spirulina platensis on Modifying Culture Media. Energy Reports, Volume 5, pp. 327-336.

[3] Soni, R. A., Sudhakar, K. & Rana, R. S., 2017. Spirulina – From growth to nutritional product: A review. Trends in Food Science & Technology, Volume 69(A), pp. 157-171.

[4] Kurniawati, R., Praharyawan, S. & Tri-Panji, 2020. Optimasi Nisbah Natrium Nitrat: Urea dan Konsentrasi Nitrogen Pada Kultivasi Spirulina platensis untuk Produksi Protein dan Pigmen Fikosianin. Menara Perkebunan, 88(2), pp. 130-140.

[5] Tietze, H., 2004. Spirulina Micro Food Macro Blessing. 4 ed. Australia: Harald W. Tietze Publishing.

[6] Goldman, J., 1979. Outdoor algal mass culture. II. Photosynthetic Yield Limitations. Water Research, 13(2), pp. 119-136.

[7] Christmadha, T., Panggabean, L. & Mardiati, Y., 2006. Pengaruh Konsentrasi Nitrogen dan Fosfor Terhadap Pertumbuhan, Kandungan Protein, Karbohidrat dan Fikosianin Pada Kultur Spirulinafusiformis. Berita Biologi, 8(3), pp. 163-169.

[8] Budiardi, T., Utomo, N. & Santosa, A., 2010. Pertumbuhan dan Kandungan Nutrisi Spirulina sp. pada Fotoperiode yang Berbeda. Jurnal Akuakultur Indonesia, 9(2), pp. 146-156.

[9] Christwardana, M., Nur, A. & Hadiyanto, H., 2013. Spirulina platensis: Potensinya Sebagai Bahan Pangan Fungsional. Jurnal Aplikasi Teknologi Pangan, 2(1), pp. 1-4.

[10] Nemerow, N. L., 1991. Stream, Lake, Estuary, and Ocean Pollution. 2nd ed. United States: s.n.

[11] Salunkhe, D. K., Kadam, S. S. & Chavan, J. K., 1985. Postharvest Biotechnology of Food Legumes. 1st ed. Florida: CRC Press.

[12] Suminto, 2009. Penggunaan Jenis Media Kultur Teknis Terhadap Produksi dan Kandungan Nutrisi Sel Spirulina platensis. Jurnal Saintek Perikanan, 4(2), pp. 53-61.

[13] Putra, R., Mercuriani, I. & Semiarti, E., 2016. Pengaruh Cahaya dan Temperatur Terhadap Pertumbuhan Tunas dan Profil Protein Tanaman Anggrek Phalanopsis anabilis Transgenik Pembawa Gen Ubipro: PaFT. Bioeksperimen, 2(2), pp. 79-90.

[14] Bui, F., Lelang, M. & Taolin, R., 2015. Pengaruh Komposisi Media Tanam dan Ukuran Polybag Terhadap Pertumbuhan dan Hasil Tomat (Licopercicum escelentum, Mill). Jurnal Pertanian Konservasi Lahan Kering, 1(1), pp. 1-7.

[15] Becker, W. et al., 1994. Microalgae, Biotechnology, and Microbiology. New York: Cambridge University Press.

[16] Natalia, Amin, B. & Effendi, I., 2019. Effect of Addition of Different Nitrate Concentration on Spirulina platensis Biomass with Semi Outdoor System. Asian Journal of Aquatic Sciences, Volume 2, pp. 127-131.

[17] Nainggolan, J. G. M., Tanjung, A. & Effendi, I., 2018. Growth of Spirulina platensis in Indoor and Semi Outdoor Culturing Systems. Asian Journal of Aquatic Sciences, 1(1), pp. 22-28.

[18] Isnansetyo, A. & Kurniastuty, 1995. Teknik Kultur Phytoplankton Zooplankton, Pakan Alam untuk Pembenihan Organisme Laut. Yogyakarta: Kanisius.

[19] Madigan, M. T., Martinko, J. M., Stahl, D. A. & Clark, D. P., 2011. Brock Biology of Microorganisms. 13th ed. San Francisco: Pearson Education Inc.

[20] Ulya, S., Sedjati, S. & Yudiati, E., 2018. Kandungan Protein Spirulina platensis pada Media Kultur dengan Konsentrasi Nitrat (KNO3) yang Berbeda. Buletin Oseanografi Marina, 7(2), pp. 98-102.

[21] Sari, F. Y. A., Suryajaya, I. M. A. & 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.

[22] Tambunan, A. L., Yuniar, I. & Trisyani, N., 2022. Kultur Pertumbuhan Mikroalga Spirulina sp. pada Media Asam, Netral, dan Alkaline Skala Laboratorium. Jurnal Perikanan dan Ilmu kelautan, 4(1), pp. 28-37.

[23] Tinambunan, J., Wijayanti, M. & Jubaedah, D., 2017. Pertumbuhan Populasi Spirulina platensis dalam Media Limbah Cair Bahan

Olahan Kecap dan Media Zarrouk. Jurnal Akuakultur Rawa Indonesia, 5(2), pp. 209-219.

[24] Oktafiani, M. & Joni, H., 2013. Pengaruh Konsentrasi Nutrien dan Konsentrasi Bakteri pada Produksi Alga dalam Sistem Bioreaktor Proses Batch. Jurnal Teknik POMITS, 2(2), pp. 57-62.

[25] Andersen, R. A., 2005. Algal Culturing Techniques. 1st ed. UK: Elsevier Academic Press.

[26] Richmond, A., 2004. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. 1st ed. Australia: Blackwell Science Ltd.

[27] Hu, Q., 2004. Environmental Effects on Cell Composition. In: In: Richmond A (ed.). Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Australia: Blackwell Science Ltd, pp. 114-122

[28] Wahidin, S., Idris, A. & Shaleh, S. R. M., 2013. The Influence of Light Intensity and Photoperiod on The Growth and Lipid Content of Microalgae Nannochloropsis sp. Bioresource Technology, Volume 129, pp. 7-11.

[29] Wijihastuti, R., Luthfiyah, A. & Noriko, N., 2020. Pengaruh Pertumbuhan Spirulina sp. Terhadap Penggunaan Pupuk Organik Cair Sebagai Media Tumbuh. Jurnal Al Azhar Indonesia Seri Sains dan Teknologi, 5(4), pp. 202-207.

[30] Widawati, D., Santosa, G. W. & Yudiati, E., 2022. Pengaruh Pertumbuhan Spirulina platensis Terhadap Kandungan Pigmen Beda Salinitas. Journal of Marine Research, 11(1), pp. 61-70.

[31] Manirafasha, E. et al., 2016. Phycobiliprotein: Potential Microalgae Derived Pharmaceutical and Biological Reagent. Biochemical Engineering Journal, Volume 109, pp. 282-296.

[32] Fretes, H., Susanto, A., Prasetyo, B. & Limantara, L., 2012. Karotenoid dari Makroalgae dan Mikroalgae: Potensi Kesehatan Aplikasi dan Bioteknologi. Jurnal Teknologi dan Industri Pangan, 23(2), pp. 221-228.

[33] Aryono, B., Zainuddin, M. & Fithria, R. F., 2022. Pertumbuhan, Kadar Pigmen dan Aktivitas Antioksidan Spirulina platensis pada Kultur dengan Perbedaan Warna Pencahayaan Leds. Journal of Marine Research, 11(4), pp. 805-818.

[34] Amir, M., Agustini, W. & Caesar, Q., 2013. Analisis Protein, Karbohidrat, Lemak, dan Pigmen Fikobiliprotein Mikroalga Spirulina platensis yang Dikultivasi pada

[35] Purba, N. E., Suhendra, L. & Wartini, N. M., 2019. Pengaruh Suhu dan Lama Ekstraksi dengan cara Maserasi terhadap Karakteristik Pewarna dari Ekstrak Alga Merah (Gracilaria sp.).

[36] Sakka, A. et al., 1999. Nitrate, Phosphate, and Iron Limitation of The Phytoplankton Assemblage in The Lagoon of Takapoto Atoll (Tuamotu Archipelago, Frnch Polynesia). Aquatic Microbial Ecology, Volume 19, pp. 149-161.

[37] Prayitno, J., 2016. Pola Pertumbuhan dan Pemanenan Biomassa dalam Fotobioreaktor Mikroalga untuk Penangkapan Karbon. Jurnal Teknologi Lingkungan, 17(1), pp. 45-52.