BIOMASS PRODUCTION OF MICROALGAE *Spirulina platensis* WITH DIFFERENT CULTIVATION MEDIA AS AN ANTIMALARIA MATERIAL

Dwi Sunu Widyartini*, Endang Ariyani Setyowati
Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia

*Email: dwi.widyartini@unsoed.ac.id

Abstract. *Spirulina platensis* microalgae contain high protein and can reach 70% of its dry weight. Other content as antioxidants to prevent the emergence of free radicals, its flour has the potential to be immunostimulant and antimalarial. The ability of this microalgae to produce various sustainable products is not yet balanced with the development industry that meets biomass needs. The purpose of this study was to determine the effect of fertilizer types on the production of *S. platensis* microalgae biomass and to determine the cultivation media that can produce the highest cell biomass. This study used an experimental method with a Completely Randomized Design. The treatments tried were the kind of fertilizers, namely grow more, KW_{21}, liquid organic fertilizer, and eco enzyme. Replications 5 times. The variables observed were the independent variables, namely the kind of fertilizer given, and the dependent variable was cell biomass. Supporting parameters were light, pH, and brightness. Biomass data were analyzed by the F-test to determine the effect of treatment and continued with the LSD test to determine the differences between treatments. The results of the study showed that the treatment of fertilizer types caused the *S. platensis* cell biomass to be different. Media with KW21 fertilizer produced the highest *S. platensis* biomass.

Keywords: biomass, fertilizer, flour, media, *Spirulina*

A. Introduction

Microalgae are biological agents that have not been widely developed by researchers in Indonesia, although microalgae have great potential to overcome various chronic problems of the modern era. These chlorophyll microorganisms play a role in photosynthesis and produce organic materials that can be utilized in various human needs, including the food industry, supplements, pharmaceuticals, and cosmetics, and can be an alternative bioenergy. Microalgae for food, until now only *Spirulina* and *Chlorella* have been recognized as food grade.

Spirulina platensis is a cylindrical microalgae with thin cell walls. The protein content of *Spirulina* is very high, reaching 55-70%, carbohydrates 15-25%, and essential fatty acids 18% of its dry weight. The amino acid content of this microalgae is quite balanced, namely isoleucine, leucine, methionine, phenylalanine, tryptophan, valine, lysine, arginine, histidine, and threonine. *S. platensis* also contains various vitamins with high concentrations, especially vitamins A and B_{12}. Provitamin-A is an antioxidant that acts synergistically with minerals, vitamins, and phytonutrients to prevent the emergence of free radicals. Minerals and several pigments such as carotene, chlorophyll, and phycobiliprotein (phycocyanin, phycoerythrin, and allophycocyanin) are high, namely 60% of the total protein. The high protein causes *S. platensis* to have the potential as an antioxidant and is also useful as an anti-inflammatory (1) (2) (3).

According to (4), *Spirulina* can be used as fish feed, functioning as an immunostimulator. Giving *Spirulina* 4 g per kg of feed is more effective in increasing the immunity of catfish.

According to (5), ethanol extract of *S. platensis* is also a promising source of antimalarial compounds, based on inhibition of PfMQO. Ethanol extract of *S. platensis* of 91.999% and 5.25 $g.mL^{-1}$ can inhibit PfMQO and IC50. These results indicate that the extract provides high antimalarial activity exceeding the theoretical standard of antimalarial bioactive compounds. Microalgae have great potential to be developed into both products, but there are still many challenges to be faced. One of them is the small number of microalgae development industries in Indonesia. According to (6), the market for microalgae-based products in 2020 reached $1547 million and is projected to double by 2028. Therefore, it is still necessary to design new functional foods, nutraceuticals, and pharmaceuticals based on microalgae, in addition to isolating single compounds from active compounds that have physiological functions and are drug candidates. To produce a product, a large and continuous biomass is needed. Therefore, it is necessary to cultivate *Spirulina* microalgae to support the provision of microalgae both in terms of quality and quantity (7). This study aims to determine the effect of different fertilizers on *Spirulina* sp. cell biomass and determine the kind of fertilizer that produces the highest *Spirulina* cell biomass in semi-mass scale culture.

B. Methods

The materials used in this study were microalgae *S. platensis*, Grow more leaf fertilizer, KW21 fertilizer, Liquid Organic Fertilizer (POC) and Eco Enzyme (EE), Vitamin B_{12}, Na_2SiO_3 (silicate), Vitamins, and Aquades. Materials for protein content analysis are kjeldalh tablets, concentrated H_2SO_4, 50% NaOH, PP, 3% boric acid, and 0.1 N HCL. The equipment used in this study is analytical scales, basins, plastic, sample bottles, gallons, stirring rods, jet pump aerators, hoses, hexane bottles, neon lights, planktonnets, Sedgewick rafters, cover glasses, hand counters, microscopes, Lux meters, plastic funnels, centrifuges, vortexes, autoclaves, ovens, refrigerators, pH-meters, salt testers, thermometers, Erlenmeyer flasks, beaker glasses, dropper pipes, plastic vials, kjeldalh flasks, titration pipettes, stoves, cotton, tissue, aluminum foil.

This study used an experimental method with a Completely Randomized Design. The treatments tried were the kind of fertilizers, namely grow more, KW_{21}, liquid organic fertilizer, and eco enzyme. Replications 5 times. The variables observed were the independent variables, namely the kind of fertilizer given, and the dependent variable was cell biomass. Supporting parameters were light, pH, and brightness. Biomass data were analyzed by the F-test to determine the effect of treatment and continued with the LSD test to determine the differences between treatments (8). *S. platensis* microalgae cultivation on various media according to treatment for making growth curves and determining optimum harvest time. A good initial density for microalgae is 2.0 x 10^5 $cells.ml^{-1}$. The biomass is then weighed with an analytical balance. The biomass of *S. platensis* microalgae cells is calculated using the formula (9).

$$\text{Biomass cells } \left(\frac{g}{mL}\right) = \frac{(W_1\text{-}W_0) \times 1000}{V}$$

Description:
W1 = Dry weight of container + cell biomass (g)
W0 = Dry weight of container (g)
V = Sample volume

The biomass data of *S. platensis* from each treatment were statistically tested using SPSS software using ANOVA at a test level of 5%. The results of the analysis were significantly or very significantly different, followed by the LSD test to determine the differences between treatments.

C. Results And Discussion

The results of *S. platensis* microalgae cultivation using different media (Figure 1) show that all treatments of *S. platensis* microalgae experienced increased growth up to the exponential phase. After the exponential phase, followed by the stationary phase, which is the phase where the growth rate becomes zero. In this phase, there is an accumulation of toxins due to microalgae metabolism, nutrient deficiencies, and changes in environmental conditions.

Figure 1. Microalgae *S. platensis* and laboratory scale culture

The number of living microalgae cells is the same as the number of dead cells. The decline in growth in all treatments also occurred again at the end of the culture which can be called the death phase. The environmental conditions of the media are still good, even the pH of the media is also alkaline. However, the nutrients contained in the media have begun to be used up by the microalgae so that they cannot grow and even die (10). The growth of *S. platensis* microalgae cells can produce different average cell biomass, between 46.00–145.85 mg.50L^{-1} (Figure 2). KW_{21} fertilizer is a fertilizer that is made and commonly used for *S. platensis* microalgae culture at BBPBAP Jepara.

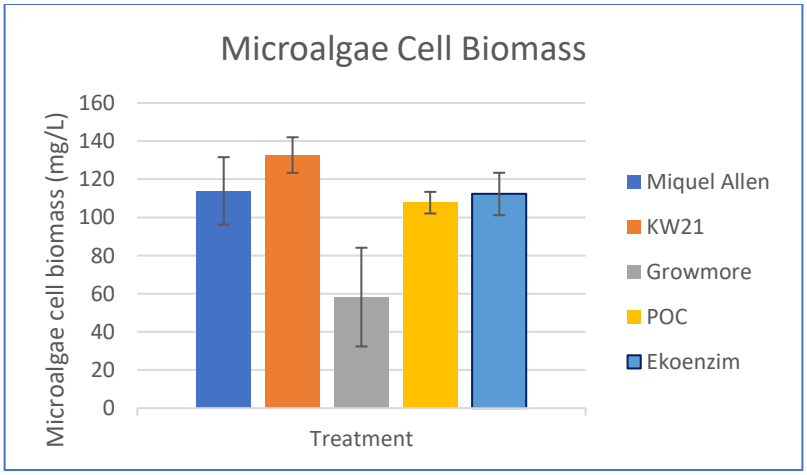

Figure 2. Mean biomass of weighing results at the end of culture

The microalgae cultivation system requires a medium that has sufficient nutrient content for its growth. The nutrients are in the form of fertilizers used for the growth and metabolism of microalgae. One solution has been found by biologists, namely by using organic waste to become Liquid Organic Fertilizer (POC) and Eco Enzyme (EE) which are very useful and can decompose the organic waste. Liquid Organic Fertilizer is a solution from the decomposition of organic materials originating from plant waste, agro-industrial waste, animal waste, and human waste that contains more than one nutrient. Eco Enzyme is the result of the fermentation

of organic kitchen waste, such as fruit and vegetable dregs, sugar (brown sugar, brown sugar, or cane sugar), and water.

POC and EE are also very environmentally friendly because they do not contain chemicals (11) (12). Grow more leaf fertilizer can be used for types of vegetables, food crops, fruits, and perennial plants. Although intended for plants, Grow more fertilizer can also be used for microalgae, (13), commercial fertilizer can be used as an alternative for microalgae growth because it has sufficient macro and micro nutrients for microalgae growth. Grow more fertilizer with a dose of 1 g/L can provide a high biomass of 1.02 g/L. The N nutrient element of grow more leaf fertilizer is 32%. The composition of Grow more content consists of the elements N (32%), P (10%), K (10%), and Mg (0.1%) and also contains micronutrients including Mn, Bo, Cu, Co, and Zn as well as vitamins for plant growth (14) (15).

This KW_{21} fertilizer is a liquid fertilizer produced abroad, so the price is relatively expensive and rarely available in the local market. This can be a problem in itself experienced by natural feed farmers. In addition to the problem of price and limited availability, the absence of silicate elements in this fertilizer is also a weakness, especially for diatom phytoplankton culture activities. This KW_{21} fertilizer is a liquid fertilizer produced abroad, so the price is relatively expensive and rarely available in the local market. This can be a problem experienced by natural feed farmers. In addition to the problem of price and limited availability, the absence of silicate elements in this fertilizer is also a weakness, especially for Diatom microalgae culture activities (16) (17).

Table 1. F-test for treatment of *S. platensis* biomass

source	sum of squares	df	mean square	f	sig.
corrected model	15470,057[a]	4	3867,514	15,791	0,000
intercept	275444,430	1	275444,430	1124,660	0,000
treatment	15470,057	4	3867,514	15,791	0,000
error	4898,271	20	244,914		
total	295812,758	25			

R Squared = 0,760 (Adjusted R Squared = 0,711)

Table 2. LSD test for treatment of *S. platensis* biomass

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Miquel Allen	KW21	-18,7900	9,89775	0,072	-39,4363	1,8563
	Growmore	55,6720*	9,89775	0,000	35,0257	76,3183
	POC	6,1700	9,89775	0,540	-14,4763	26,8163
	Ekoenzim	1,6200	9,89775	0,872	-19,0263	22,2663
KW21	Miquel Allen	18,7900	9,89775	0,072	-1,8563	39,4363
	Growmore	74,4620*	9,89775	0,000	53,8157	95,1083
	POC	24,9600*	9,89775	0,020	4,3137	45,6063
	Ekoenzim	20,4100	9,89775	0,052	-,2363	41,0563
Growmore	Miquel Allen	-55,6720*	9,89775	0,000	-76,3183	-35,0257
	KW21	-74,4620*	9,89775	0,000	-95,1083	-53,8157
	POC	-49,5020*	9,89775	0,000	-70,1483	-28,8557
	Ekoenzim	-54,0520*	9,89775	0,000	-74,6983	-33,4057
POC	Miquel Allen	-6,1700	9,89775	0,540	-26,8163	14,4763
	KW21	-24,9600*	9,89775	0,020	-45,6063	-4,3137
	Grow more	49,5020*	9,89775	0,000	28,8557	70,1483
	Eco-enzim	-4,5500	9,89775	0,651	-25,1963	16,0963
Eco-enzim	Miquel Allen	-1,6200	9,89775	0,872	-22,2663	19,0263
	KW21	-20,4100	9,89775	0,052	-41,0563	,2363
	Grow more	54,0520*	9,89775	0,000	33,4057	74,6983
	POC	4,5500	9,89775	0,651	-16,0963	25,1963

The error term is Mean Square (Error) = 244,914.
*. The mean difference is significant at the 0,05 level.

The results of the analysis of *S. platensis* biomass data with statistical testing produced an F-test for treatment less than 0.01, which means that there is a very significant difference in the biomass of *S. platensis* treatment (the treatment is very different)

In the results of the LSD test, the significance value is below 0.05, meaning it is significantly different, the part is the significance value below 0.05. Grow more is significantly different when compared to other treatments.

Microalgae generally live well at neutral pH (pH 7). According to (34), photosynthesis activity will decrease to a maximum of 33% when the pH drops to 5.0. Water is the main component of protoplasm and plays an important role in cell metabolism. Water has several functions in the body as a solvent and a means of transporting nutrients needed by the body. Acidic waters with a pH of less than 6.0 can cause microalgae to not live well. Waters with a pH value of less than 4.0 and more than 9.5 can reduce the productivity of aquatic organisms including microalgae.

Extract of *S. platensis* has the potential as an anticancer that can inhibit breast cancer cells and is safe for normal cells. Biomass and phycocyanin pigment from *S. fusiformis* can reduce blood glucose levels in diabetic rats. Meanwhile, for the cosmetic industry, a face mask made from *Spirulina* and fish collagen can be made that can inhibit acne-causing bacteria. Phycocyanin pigment, crude extract, and alkaloid fraction from *S. platensis* can inhibit *Plasmodium falciparum* (malaria virus) (1) (2).

D. Conclusion

The kind and concentration of culture media fertilizer caused the results of *S. platensis* cell biomass to be different. Media with KW_{21} fertilizer produced the highest *S. platensis* biomass. Research is still needed to obtain the optimum concentration of media with KW_{21} fertilizer as a targeted discovery media for microalgae culture. Media with KW_{21} fertilizer Media with KW_{21} fertilizer which can produce high protein for antimalarials.

E. Acknowledgement

Thanks to LPPM with FTGB - BLU Contract number 26.744/UN23.35.5/PT.01/II/2024, dated February 26, 2024.

F. References

[1]. García, J.L, de Vicente M, Galán B. (2017) Microalgae, Old Sustainable Food and Fashion Nutraceuticals. *Microb Biotechnol*, vol. 10, no. 5, pp. 1017–24.

[2]. Finamore, A., Palmery, M., Bensehaila, S., Peluso, I. (2017) Antioxidant, Immunomodulating, and Microbial-Modulating Activities of The Sustainable and Eco-Friendly *Spirulina*. Oxid Med Cell, Longev.

[3]. Bioalgae (2022) *Spirulina* dapat meningkatkan daya tahan. www.researchgate.net/publication/40429949_Spirulina_ameliorates_immunity_and_reduces_viral_load_in_beta_halassemia_major_children_comorbid_with_hepatitis_virus_C_A_single-arm_clinical_trial_Medical_Science_Article.: (November 16).

[4]. Simanjuntak, S.B.I., Wirawidjaja, A.M., Dana, D., Supriyadi, H. (2012) Efektivitas *Spirulina* Sebagai Immunostilulan Pada Ikan Patin Jambal (*Pangasius djambal*). *J Biol Indon.*, vol. III, no. 3, pp. 209–218.

[5]. Setyowati, E.A., Isnansetyo, A., Djohan, T.S., Nurcahyo, R.W., Prabandari, E.E. (2020) Antimalarial Activity of Microalgae Extracts Based on Inhibition of PfMQO, A Mitochondrial PhCOGJ. *Plasmodium falciparum* enzyme. *Pharmacog J.*, vol. 11, no. 6, pp.1477–82.

[6]. Setyaningsih, I. (2022) Paparan Manfaat *Spirulina*, Dari Anti Jerawat Hingga

Antikanker. PPID IPB. (May 19).

[7]. Wardani, N.K., Supriyartini, E., Santosa, G.W. (2022) Pengaruh Konsentrasi Pupuk Walne Terhadap Laju Pertumbuhan dan Kandungan Klorofil-a *Tetraselmis chuii*. *Journal Of Marine Research.*, vol. 11, no. 1, pp. 77–85.

[8]. Steel, R.G.D., Torrie, J..H. (1993) Prinsip dan Prosedur Statistika (Pendekatan Biometrik). Gramedia Pustaka. Utama, Jakarta.

[9]. Fakhri, M., Wisnu, Ekawati, A.W. (2020) Pengaruh Salinitas Terhadap Pertumbuhan, Biomassa dan Klorofil-A *Dunaliella* sp. *Journal of Fisheries and Marine Research.*, vol. 4, no. 3, pp. 395-398.

[10]. Colman, B., Gehl, K.A. (1983) Effect of External pH on The Internal pH of *Chlorella saccharophila*. *J Plant Physiol.*, vol. 77, no. 4, pp. 917-921.

[11]. Budiardi, T., Nur, B.P.U., & Asep, S. (2010) Pertumbuhan dan Kandungan Nutrisi *Spirulina* sp. pada Fotoperiode yang Berbeda. *Jurnal Akuakultur Indonesia*, vol. 9, no. 2, pp. 146-156.

[12]. Kawaroe, M., Prartono, T., Rachmat, A., Sari, D.W., Agustine, D. (2012) Laju Pertumbuhan Spesifik dan Kandungan Asam Lemak Pada Mikroalga *Spirulina platensis*, *Isochrysis* sp. dan *Porphyridium cruentum. Ilmu Kelautan*, vol. 17, no. 3, pp. 125–131.

[13]. Selvia, N. (2018) Pupuk organik cair dan eco enzyme selamatkan bumi. *Buletin Oseanografi Marina*. vol. 7, no. 52, pp. 98–102.

[14]. Prayitno, J. (2015) Pemberian Pupuk Komersial Untuk Pembentukan Biomassa Pada Kultur Mikroalga *Chlorella* sp. Dalam Sistem Fotobioreaktor Penangkap Karbon. *Jurnal Teknologi Lingkungan*,vol. 16, no. 1, pp. 37–42.

[15]. Prayitno, J. (2016) Pola Pertumbuhan dan Pemanenan Biomassa Dalam Fotobioreaktor Mikroalga Untuk Penangkapan Karbon. *Jurnal Teknologi Lingkungan*. vol. 17, no. 1, pp. 45–52.

[16]. Ulya, S., Sedjati, S., Yudiati, E. (2018) Kandungan Protein *Spirulina platensis* Pada Media Kultur Dengan Konsentrasi Nitrat (KNO_3) Yang Berbeda. *Buletin Oseanografi Marina.* vol. 7, no. 2, pp. 98–102.

[17]. Widayat & Hardiyanto. (2015) Pemanfaatan Limbah Cair Industri Tahu Untuk Produksi Biomassa Mikroalga *Nannochloropsis* sp. Sebagai Bahan Baku Biodiesel. *Reaktor*. vol. 15, no. 4, pp. 253–260.

[18]. Yulia, F.G., Zuhry, E. (2022) Respon Berbagai Konsentrasi dan Interval Pupuk Daun *Growmore* Terhadap Pertumbuhan dan Produksi Tanaman Kangkung Darat (*Ipomea reptans* Poir). *Jom Faperta*. vol. 9, no. 1, pp. 1–14.