Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology
Available at http://ejournal.undip.ac.id/index.php/saintek
Vol. 19 No. 3 : 143 - 151, October 2023

EFFECT OF COMBINATION INORGANIC FERTILIZER WITH RICE WASHING WATER ON THE NUTRITIONAL CONTENT OF *Spirulina* sp.

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Received: 17 August 2023, Accepted: 6 October 2023

ABSTRACT

Spirulina sp. culture. usually use growth fertilizers such as walne, zarrouk, or guillard micro and macronutrient content has been balanced. However, the use of this fertilizer has several obstacles, including the price being relatively expensive, and requiring quite a lot of nutrient solution composition to make. This research aims to provide information regarding the use of a combination of inorganic fertilizer from urea, plant catalyst, and GA with rice washing water as an alternative fertilizer that has high nutritional content and can be used for *Spirulina* sp. The results showed that the combination of inorganic fertilizer with rice washing water had a positive influence on the culture of Spirulina sp. The results of optical density analysis and wet biomass yield showed an increase compared to K- (without fertilizer). The results of another analysis of a combination of inorganic fertilizer and rice washing water produced the highest protein value in treatment P2 with a value of 5.42%. Meanwhile, the highest fat content was in the P3 treatment with a value of 1.96%.

Keywords: Inorganic fertilizer; rice washing water; *Spirulina* sp.

INTRODUCTION

Spirulina sp. is a fishery commodity originating from a group of cyanobacterium-type microalgae with autotrophic, multi-cellular, prokaryotic, and filamentous properties and between 50-500 µm in length and 3-4 µm in width (Jung *et al.* 2019). These microalgae have great potential to be used as a source of functional food, nutraceuticals, cosmetics, and pharmaceuticals because they have many nutrients that can be utilized (Castro *et al.* 2019; Jung *et al.* 2019). The nutrients contained in *Spirulina* sp., based on Liestianty *et al.* (2019) and Jung *et al.* (2019) namely 64.24% protein, essential and non-essential amino acids, 6–8% fat (heptadecanoic, stearic, oleic, palmitoleic, omega-3, omega-6, myristic fatty acids, linoleic acid (LA) gamma acids linoleic (GLA), and palmitate), carbohydrates 15–25%.

Utilization of this potential needs to be supported by the availability of *Spirulina* sp. by carrying out cultural activities in controlled conditions. *Spirulina* sp. is a type of microalgae that can be easily cultured by paying attention to the nutrition in the culture medium. Utomo *et al.* (2020) explain the nutritional composition of fertilizer that is good and balanced when cultivating *Spirulina* sp. can determine the nutritional content of this microalgae. The type of fertilizer is usually used in *Spirulina* sp. culture. are walne, guillard, and zarrouk. This fertilizer has standardized nutritional content, both micro and macronutrient content, according to what is needed for the growth of *Spirulina* sp. In its application, this third fertilizer has weaknesses, namely the price is relatively expensive and the composition required is quite large (Sirait *et al.* 2019; Utomo *et al.* 2005; Viqran *et al.* 2018).

Several studies have been conducted regarding alternative fertilizers for *Spirulina* sp. including Leksono *et al.* (2017) informed that the use of fermented liquid organic fertilizer from *Azolla pinnata* on *Spirulina* sp. The nutritional value was obtained from the treatment with the greatest concentration, namely 47% protein, 4.6% fat, 9% ash, 34%, and 10% crude fiber. Utomo *et al.* (2020) stated that the growth rate of *Spirulina* sp. the best was at a concentration of 1 mL/L with rice washing water fertilizer of 0,57 ± 0,001 cells/day. Amanatin and Nurhidayat (2013) reported that *Spirulina* sp. cultured using a combination of urea and bean sprout extract (MET) produced the highest protein content in the treatment of a composition of 100 ppm urea and 4% MET, with a protein content of 20,997%. Based on this background, this study intends to provide information regarding the use of a combination of inorganic fertilizers from urea, plant catalyst, GA, and rice washing water as an alternative fertilizer that has a high nutritional content and can be used for *Spirulina* sp. cultures.

RESEARCH METHODS

Material

The main ingredients used are *Spirulina* sp., gibberellic acid (GA) organic fertilizer, plant catalyst fertilizer, urea fertilizer, first rice washing water, 15 ppt seawater, filter paper, distilled water, and freshwater. The tools used are elemeyer (Iwaki), 400 mesh nylon fabric, culture container with a capacity of 5 L, tubular lamp with 3000 lux intensity, aerator (500-AP), UV-vis RS UV 2500, refrigerator (Sharp), ultraviolet lamp (Submersible Aquarium UV Sakkai Pro 45 watt)

Method

Culture of *Spirulina* sp. using a combination of inorganic media (plant catalyst fertilizer, urea, and gibberilic acid) and rice washing water referring to Astriandari research (2018) with modifications added are the fertilizer and photoperiod of lighting used. Culture of *Spirulina* sp. is carried out in a container with a capacity of 5 L containing seawater with a concentration of 15 ppt as much as 1 L with a seed concentration of 20% and additional fertilizer media according to the treatment (the treatment can be seen in table 1). During the culture process, *Spirulina* sp. was given irradiation with a tubular lamp at a capacity of 3000 lux and aeration for 24 hours. Harvesting is carried out if the optical density value is above ≥0.5. The harvesting process was carried out by filtering the culture and rinsing it using distilled water. Each culture treatment was given three repetitions.

Table 1. Combination of Inorganic Fertilizers and Rice Washing Water

Perlakuan/Treatment	Pupuk/Fertilizer
K- (Kontrol Negative/ Negative Control)	No fertilizer
K+ (Kontrol Positif/ Positive Control)	Walne fertilizer 0.5 mL
P1	1% inorganic fertilizer and 1 mL/L rice washing water
P2	1% inorganic fertilizer and 3 mL/L rice washing water
P3	1% inorganic fertilizer and 5 mL/L rice washing water
P4	1% inorganic fertilizer and 7 mL/L rice washing water
P5	1% inorganic fertilizer and 9 mL/L rice washing water

Optical Density *Spirulina* sp.

The optical density (OD) value was based on (Afriani *et al.* 2018) measured using a spectrophotometer to see the growth of *Spirulina* sp. at a wavelength of 670 nm every day until the OD value is ≥0.5.

Yield of *Spirulina* sp. Wet Biomass

The culture of *Spirulina* sp. was filtered using 400 mesh nylon pores and then rinsed using distilled water. Wet biomass was weighed to determine the weight of the resulting yield.

Water Quality Analysis

The quality of *Spirulina* sp. culture water. including temperature, pH, and salinity were analyzed every day until the harvesting process was carried out. The harvesting process will be carried out if the optical density of *Spirulina* sp. has reached ≥0.5.

Moisture Content Analysis (AOAC 2005)

The water content of *Spirulina* sp. wet biomass. obtained through calculations using the following formula:

$$\text{Moisture }(\%) = \frac{B-C}{B-A} \times 100\% \text{ ....(1)}$$

Information: A = empty cup weight (g); B = weight of the cup filled with samples (g); C = weight of the cup with the sample that has been dried (g)

Ash Content Analysis (AOAC 2005)

The Ash content of the wet biomass of *Spirulina* sp. was obtained using the dry ashing method. The principle of this analysis is by burning the sample in a furnace at 600˚C for 7 hours until the ash turns white. The ash content of *Spirulina* sp. is obtained by using the formula:

$$\text{Ash Content }(\%) = \frac{a-b}{c} \times 100\% \text{ ....(2)}$$

Information:a = weight of cup and final sample; b = cup weight; c = initial sample weight

Fat Content Analysis (AOAC 2005)

Spirulina sp. wet biomass analyzed fat content based on the AOAC method (2005) with the calculation formula after analysis is:

$$\text{Fat Content }(\%) = \frac{a-b}{c} \times 100\% \text{ ....(3)}$$

Information: a = weight of final flask and sample (g); b = fat flask weight (g); c = initial sample weight (g)

Protein Content Analysis (AOAC 2005)

Spirulina sp. wet biomass protein analysis process. carried out with the stages of destruction, distillation, and titration with the final result calculated using the formula:

$$\text{Kadar N }(\%) = \frac{(\text{mL HCl sampel} - \text{mL HCl blanko}) \times \text{N HCl} \times 14.007 \times 100}{mg\ sampel} \times 100\% \text{ ......(4)}$$

$$\text{Kadar protein }(\%) = \%\text{N} \times \text{faktor konversi } (6{,}25) \times 100\% \text{ ......(5)}$$

Carbohydrate Content Analysis

Analysis of carbohydrate content using the by-difference method or the results of reducing 100% ash content, water content, fat content, and protein content. The by-difference method can use the following formula:

Carbohydrate Content (%) = 100% - (P+KA+A+L)…(6)

Information: P = protein content; KA = moisture content; A = ash content; L = fat content

RESULT AND DISCUSSION

Optical Density *Spirulina* sp.

Spirulina sp. In this study, it was cultured in the laboratory using a combination of different growth fertilizers, namely inorganic fertilizers (urea, plant catalyst, and GA) with rice washing water to see the nutritional and phycocyanin content. The growth that occurs in *Spirulina* sp. Qualitatively it can be seen through the color change in the culture medium from light green to dark green which indicates an increase in the population. Muyassaroh *et al.* (2018) explained that one of the peak population indicators is the color change in the culture media and the population size based on growth patterns. The color on the culture of *Spirulina* sp. in this study can be seen in Figure 1, where at the beginning of the culture process until the harvest there has been a change in color from light green to dark green. Optical density (OD) measurements were also carried out in this study to determine the development of the density of *Spirulina* sp. biomass cells using spectrophotometry (Figure 2).

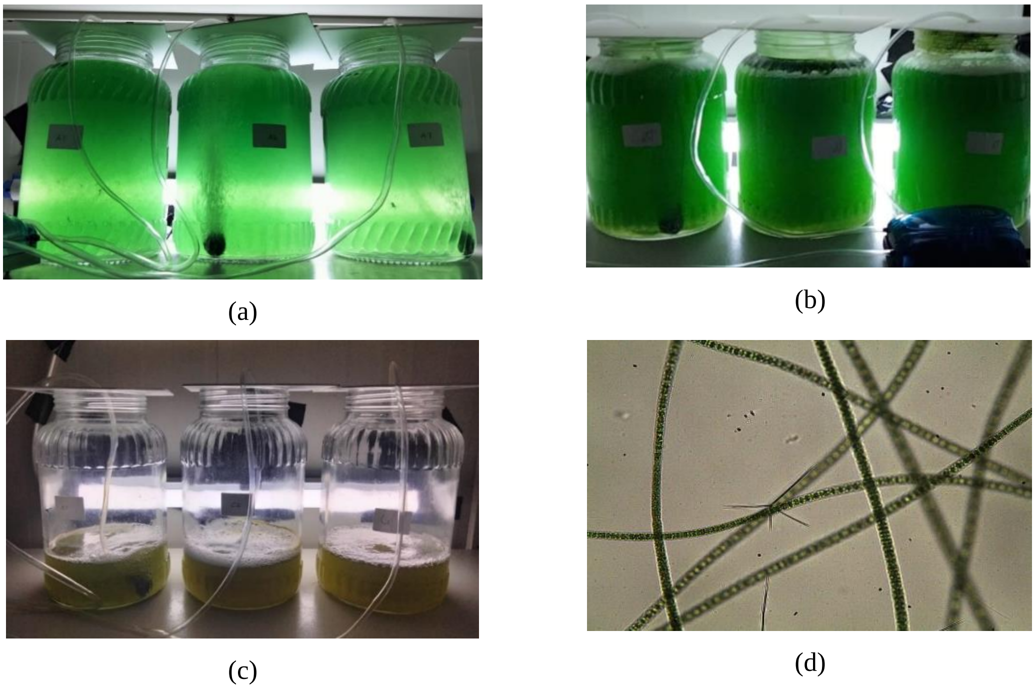

Figure 1. *Spirulina* sp.: (a) Culture of *Spirulina* sp. 1st day; (b) Culture of *Spirulina* sp. Day 5; (c) Culture of *Spirulina* sp. 8th Day; (d) *Spirulina* sp. Cells. 100x Magnification on a Light Microscope on Day 1;

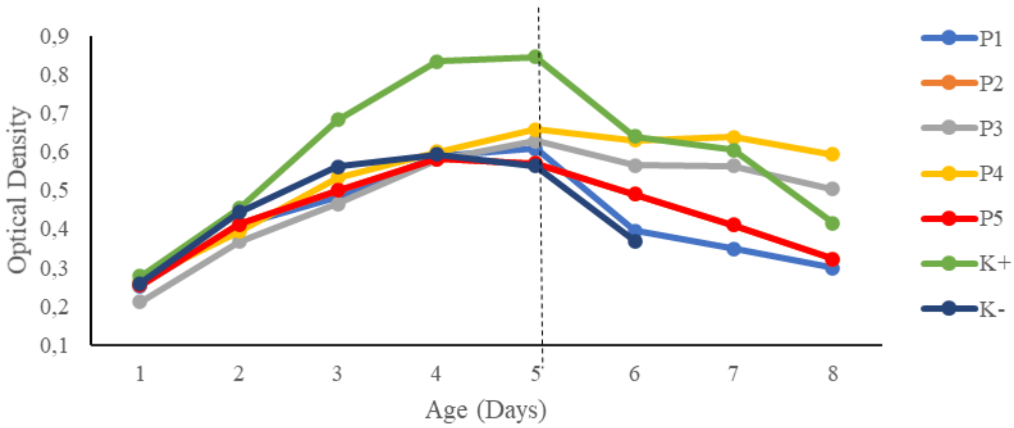

Figure 2. Optical Density Value of Spirulina sp.

The difference in nutrient administration according to the treatment resulted in different optical densities starting from the 1st day to the 8th day of observation. On day 1 the P4 treatment had the highest optical density value among the other treatments 0,2647. The OD value is not too far away compared to the K+ 0.277 treatment. The final check of the optical density value on *Spirulina* sp. was on the 8th day with the highest optical density value of 0,59 still in the P4 treatment. Based on the conclusions from the data, day 5 is the final exponential phase with the highest peak in each treatment. Lesmana *et al.* (2019) explained that the general pattern of growth for algae is divided into 4 phases, namely the lag phase, the exponential phase, the stationary phase, and the death phase.

The data provide information that there has been a difference in the value of optical density in the growth of *Spirulina* sp. cultured in the laboratory, this is due to the different nutrient composition given to each treatment. Utomo *et al.* (2020) added that the high or low value of the growth rate of *Spirulina* sp. is because there is a different nutrient content in each treatment which is used as an energy source for the growth of *Spirulina* sp.. The use of inorganic fertilizers (Urea, Plant Catalyst, GA) and rice washing water is a combination of fertilizers suitable for *Spirulina*. sp. This is because each fertilizer given to *Spirulina* sp. provides different benefits to support its growth.

GA fertilizer can increase the content of phycobiliprotein and accelerate the growth of *Spirulina* sp. biomass. (Mansouri and Telebizadeh 2015). Urea fertilizer is a chemical fertilizer containing high levels of nitrogen (N) (Yunaning *et al.* 2022), and plant catalyst is a complementary fertilizer containing phosphorus needed by *Spirulina* sp., this fertilizer can function as a catalyst to optimize the absorption of nutrients such as nitrogen and potassium from other fertilizers and can help increase the resistance of *Spirulina* sp. (Guntoro *et al.* 2017; Warganegara *et al.* 2015). Rice washing water is an organic fertilizer that has not been widely used as a microalgae fertilizer. Wijiyanti *et al.* (2019) stated that rice washing water contains many nutrients dissolved in it including protein, 80% vitamin B1, 50% phosphorus, and 60% iron which can be beneficial for the growth of *Spirulina* sp.

The data obtained stated that the harvesting of *Spirulina* sp. for all treatments it can be done on day 5. The graph shows day 5 of *Spirulina* sp. culture. is at the end of the exponential period. Ulya *et al.* (2018) reported that harvesting carried out at an exponential period would affect the process of amino acid biosynthesis. Over the exponential period, microalgae produce relatively the same amount of protein. Setyaningsih *et al.* (2013) explained that the beginning of the stationary phase (end of the exponential phase) is the end of cultured biomass production. Lesmana *et al.* (2019) provided information about the growth of *Spirulina* using catfish wastewater media, where the best treatment from their research was P3 with an exponential ending time of day 7. This period was longer than research conducted using a combination of inorganic fertilizers and rice washing water.

Water Quality Culture of *Spirulina* sp.

The growth of *Spirulina* sp. can be affected by water quality parameters. During the implementation of this research, water quality parameters were measured including temperature, pH, and salinity. Parameters of water quality for *Spirulina* sp culture. with combined inorganic fertilizers (urea, plant catalyst, GA) and rice washing, water can be seen in Table 3.

Table 3. Average Water Quality Parameters for Each Treatment When Culturing *Spirulina* sp.

Day To-	Temperature (°C)	**pH**	Salinity
0	26±0,75	7,92±0,07	
1	31±0,98	8,16±0,06	
2	30 ±1,20	8,61±0,15	19±0,83
3	29 ±0,97	8,69±0,07	
4	30 ±0,90	8,71±0,04	
5	29 ±0,91	8,71±0,18	

Temperature measurements in this study were carried out for 5 days until the end of the *Spirulina* sp. culture process. On the first day, the temperature was 26°C, a fairly low temperature for *Spirulina* sp. This is because the culture room uses air conditioning. On days 2 to 5 the temperature in the laboratory is under controlled conditions without using air conditioning, so it is relatively uniform with the measurement results tending to be constant at 30°C. In addition to air conditioning, the light used during culture is also thought to affect the temperature of the culture water. Bangun *et al.* (2015) explained that under laboratory conditions, changes in water temperature in *Spirulina platensis* culture were affected by room temperature and light intensity. Soni *et al.* (2019) reported that *Spirulina* grew better at 30°C compared to temperatures below 25°C and above 35°C. Jesus *et al.* (2018) added that the optimum temperature for the growth of *Spirulina* sp. is between 30 and 35 °C.

The pH value in the study from day 1 to day 5 increased from 7,92 to 8,71. This increase in value is thought to be due to photosynthetic activity and the decomposition of proteins and nitrogen compounds carried out by *Spirulina* sp. (Utomo *et al.* 2020; Amanatin, 2013). The pH value of the measurement results on the culture in this study is optimal for the growth of *Spirulina* sp. Hasim *et al.* (2022) reported that 7,5-9,5 is the optimum pH range for the growth of *Spirulina* sp. However, *Spirulina* sp. can still grow in culture water with a pH of 11. Cifferi (1983) stated that a good pH for the growth of *Spirulina* sp. ranges from 7-11.

Culture of *Spirulina* sp. in this study, a mixture of seawater and freshwater was used as a growing medium to obtain a salinity value with an average range for each treatment of 19 ± 0,83. This value is the value for brackish water salinity. Hasim *et al.* 2022 explain that water with a salinity lower than the average normal seawater salinity (<35 ppm) and higher than 0,5 ppm which occurs due to mixing between seawater and freshwater both naturally and artificially will become brackish water. The salinity value is still within the tolerance limit for *Spirulina* sp. growing media. According to Isnansetyo and Kurniastuty (1995), the salinity content for the growth of *Spirulina* sp. ranges from 0–35 ppt. In general, *Spirulina* sp. including microalgae which have euryhaline properties and have a considerable tolerance to changes in salinity with the ability to adapt to a wide range (Hasim *et al.* 2022; Wahyuni *et al.* 2018).

Biomass yield of *Spirulina* sp.

The yield of wet biomass of *Spirulina* sp. highest was in the K+ (walne fertilizer) at 4,80 grams, while the lowest yield was in the K- treatment (without fertilizer) with a yield of 2,63 grams. The use of combination media between inorganic fertilizers and rice washing water affects the research results. P4 is the treatment with the highest yield of 3,97 grams in 1 L of *Spirulina* sp. culture. The amount of yield was different in the treatment because the nutrient content with the combination fertilizer affected the growth of *Spirulina* sp. especially the content of N (nitrogen).

Culture media nutrients on K+ and P4 have nitrogen content from different sources. K+ comes from walne fertilizer, and P4 comes from the content of urea and rice washing water given. Walne fertilizer contains inorganic nitrogen which dissolves in water in high concentrations so that the N elements in the container are spread evenly. Urea is a fertilizer with a high inorganic nitrogen content of 45-46%, is hygroscopic, dissolves easily in water, and can react quickly as a source of nutrition when used, while rice washing water contains organic N in the form of urea (Kogoya *et al.,* 2018). Wijiyanti *et al.* (2019) explained that fresh rice washing water contains a high amount of dissolved N components. Sudartini *et al.* (2020) reported that rice washing water contained 0,29% total N, 0,05 ppm total P, 0,01 ppm total K, 1,65 ppm Ca, 7,8 ppm Mg, 0,05 mg/100g Thiamine B1.

The content in rice washing water is good for use as fertilizer in *Spirulina* sp. culture, however, adding rice washing water with an incorrect concentration can inhibit the growth of

Spirulina sp. The concentration of rice washing water in the culture medium affects the growth of *Spirulina* sp. Based on the results of the data, P5 with the addition of 9 mL/L of rice washing water had the lowest biomass than the other treatments. This is by research conducted by Utomo *et al.* (2020) which stated that using too much rice washing water affects water quality so it will inhibit the growth of *Spirulina* sp.

Protein Content

The results of the analysis of protein content in *Spirulina* sp. with a combination of inorganic fertilizers (urea, plant catalyst, and GA) and rice washing water is presented in Figure 4. The highest protein content was in the K+ treatment (walne fertilizer) which was 5,69%, but not much different from the P2 treatment (1% inorganic fertilizer and 3 mL/L of rice washing water) which has a protein content of 5,42%. This indicates that the combined fertilizer treatment in the study had a positive effect on protein content in *Spirulina* sp. wet biomass. Sirait *et al.* (2019) in their journal stated that Walne media has two sources of nitrogen in its composition, namely ammonium ions (NH4+) and nitrate ions (NO3-). Wardani *et al.* (2022) added that nitrogen is needed as a building block for protein in microalgae cells.

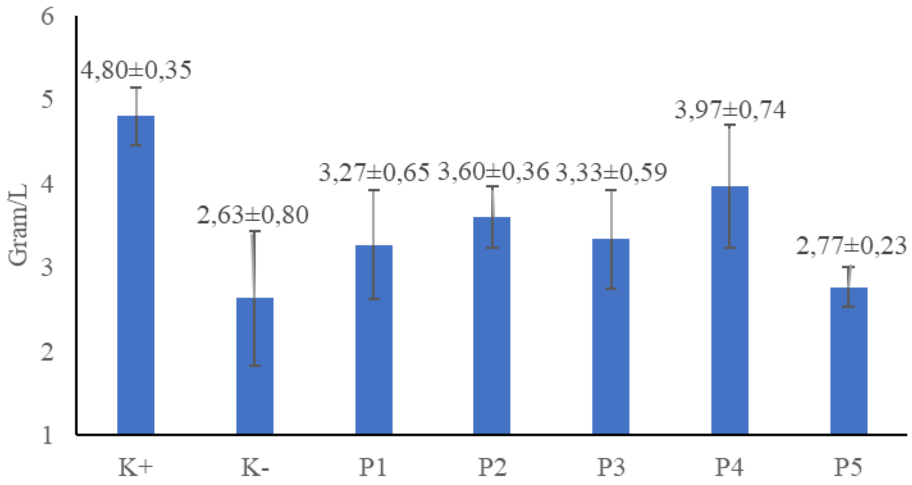

Figure 3. Yield of Wet Biomass of *Spirulina* sp.

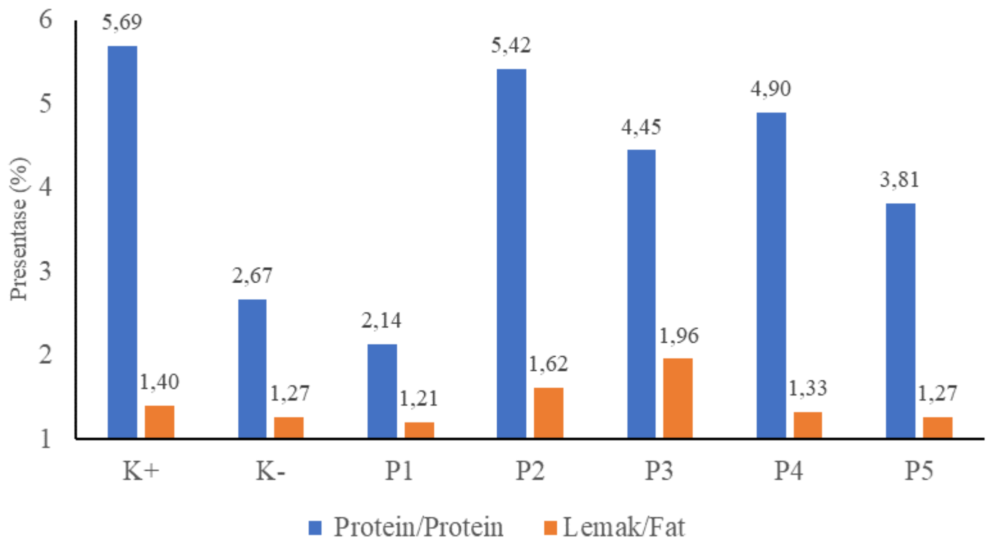

Figure 4. Protein and Fat Content of Wet Biomass Culture of *Spirulina* sp.

The treatment given in the study gave different levels of nutrients to the cultured *Spirulina* sp, especially in terms of fat and protein content. Urea inorganic fertilizer and rice washing water have a high nitrogen content. According to Herawati and Hutabarat (2014), the nutrient that influences protein and fat content is nitrogen. If nitrogen availability is limited, it will cause a decrease in protein levels (Benavente Valdes *et al.* 2016).

Fat Content

The results of the analysis of fat content in the wet biomass of *Spirulina* sp. which were cultured on a combination of inorganic fertilizers (urea, plant catalyst, GA) and rice washing water are presented in Figure 4. The highest fat content was in treatment P3 (1% inorganic fertilizer and 5 mL/L rice washing water) at 1,96%. These results are above the positive and negative control treatments which indicate the influence of the fertilizer given, according to Utomo *et al.* (2020) that the addition of rice washing water to the culture media has a positive effect on the growth of *Spirulina* sp.

The nutrient content contained in the combination media of inorganic fertilizers and rice washing water contains important elements for the growth of *Spirulina* sp. namely nitrogen and phosphorus. Nitrogen in the cultivation medium is moved directly into the cells through ATPase stimulation from Cl before the process of assimilation occurs to form amino acids combined to become a macromolecule or protein increased (Herawati and Hutabarat 2014).

Water Content

Testing the water content contained in a food product absolutely must be carried out (Daud *et al.* 2019). Based on the data obtained from the analysis of water content, the largest was found in the P3 treatment with a concentration of 1% inorganic fertilizer and 5 mL/L of rice washing water, namely 90,06%, while the positive and negative controls were below it, namely 88,59% and 88,15. %. The water content of *Spirulina* sp. wet biomass. cultured with a nutrient combination of inorganic fertilizers (urea, plant catalyst, GA) and rice washing water with several different concentrations is presented in Figure 5.

Water content is the amount of water stored in the material, either in the pores or deeper parts (Prasetyo *et al.* 2019). The cause of the high water content contained in *Spirulina* sp. is an analysis carried out on wet biomass samples and the respiration process when the culture is carried out. In the respiration process, gas exchange occurs between oxygen and carbon dioxide which produces water and other substances so a side effect of this process is the accumulation of water between cells (Murtiwuladari *et al.* 2020).

Ash Content

Ash content is an inorganic substance left over from the combustion of an organic material. The ash content and composition depend on the type of material and the method of ignition. Ash content is related to the mineral content of a material (Sudarmaji *et al.* 1989). The increase in ash content is in line with the increase in mineral content in *Spirulina* sp. Minerals play a role in maintaining osmotic pressure within the cell (Richmond 1988). The Ash content of wet biomass of *Spirulina* sp. cultured with a nutritional combination of inorganic fertilizers (urea, plant catalyst, GA) and rice washing water is presented in Figure 6.

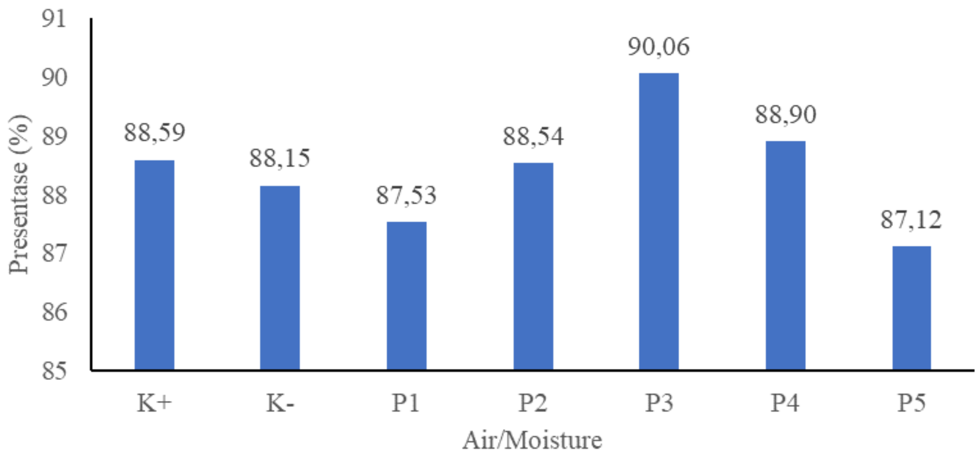

Figure 5. Water Content of Wet Biomass Culture of *Spirulina* sp.

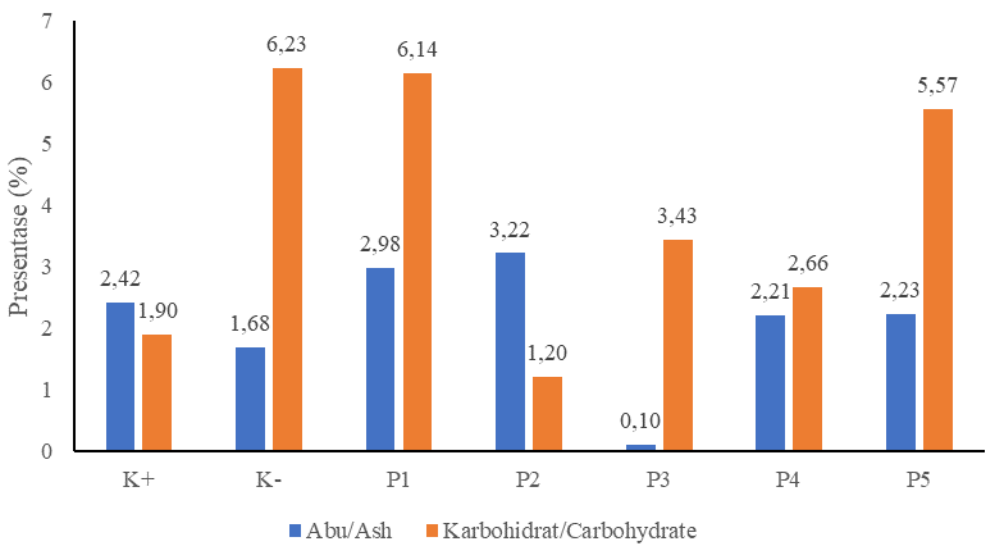

Figure 6. Ash and Carbohydrate Content of Wet Biomass Culture of *Spirulina* sp.

The results of the research showed that the results of the ash content in each treatment of the combination of inorganic fertilizers (urea, plant catalyst, GA) and rice washing water were different, the highest ash content was obtained in the P2 treatment, namely 3,22 and the lowest ash content in the P3 treatment, namely 0,10. The difference in the results of the ash content in each treatment is thought to be due to the influence of different fertilizers and the cultivation media. Hu (2004) suggested that environmental factors such as light, temperature, nutrients, and salinity not only affect photosynthesis and the productivity of cell biomass but can affect the shape and flow of cellular metabolic activities which have an impact on the dynamics of cell composition.

Carbohydrates Content

The carbohydrate content in *Spirulina* sp. which was cultured on a combination of inorganic fertilizers (urea, plant catalyst, GA) and rice washing water with several different treatments is presented in Figure 6. The highest carbohydrate content was in the K- treatment (without using fertilizers), namely 6,23%, but not much different from treatment P1 (1% inorganic fertilizer and 1 mL/L rice washing water) with a carbohydrate content of 6,14%. Meanwhile, the lowest carbohydrate content was in treatment P2 (1% inorganic fertilizer and 3 mL/L of rice washing water), namely 1,20%. according to Lebeharia (2016), carbohydrate content depends on the reduction factor of water, ash, protein, and fat content. Therefore, carbohydrates are strongly influenced by the content of supporting nutrients (moisture content, ash, protein, fat) in them. Low levels of carbohydrates can also occur if the fertilizer used in the culture of *Spirulina* sp. has a high nitrogen value. If the nitrogen content is low, the protein content will decrease and the carbohydrate content will increase, and vice versa (Sassano *et al.* 2010).

The fertilizer treatment used affected the carbohydrate content of *Spirulina* sp. wet biomass. Ocean *et al.* (2020) in a study calculated the biomass content of *Spirulina platensis* cultured using zarrouk media containing lower carbohydrates compared to *Spirulina platensis* cultured using quail droppings media with a concentration of 50 g/l. This is because, in low nitrogen conditions, it reduces the protein content to 20% and can increase the carbohydrate content to 65%.

CONCLUSION

The combination of inorganic fertilizer (Urea, plant catalyst, GA) and washing water used in the research had a positive influence on the culture of *Spirulina* sp. The results of optical density analysis and wet biomass yield showed an increase with the combination of inorganic fertilizer with rice washing water compared to K-. The results of another analysis of the treatments given produced the highest protein and fat values in treatments P2 and P3 with values of 5.42 and 1.96%.

ACKNOWLEDGEMENT

The researcher would like to express his deepest gratitude to the Institute for Research and Community Service (LPPM), Sultan Ageng Tirtayasa University for providing support and financial assistance for research through the Beginner Lecturer Research Program (PDP).

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