

Protein Content of *Spirulina* sp. Cultured Using a Combination of Urea and TSP Fertilizers

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Abstract

This study aims to determine the effect of the combined use of Urea and TSP fertilizers on the Protein content of *Spirulina* sp. This research was conducted for 10 days at the Center for Brackish Water Aquaculture (BPBAP) Takalar. The method used in this study is an experimental design with Completely Randomized Design (CRD) using 4 treatments with 3 replications, namely Treatment A (0.4 g/l Urea + 0.008 g/l TSP), B (0.5 g/l Urea + 0.010 g/l TSP), C (0.6 g/l Urea + 0.012 g/l TSP) and D (0.4 g/l Urea + 0.008 g/l TSP). The data obtained were analyzed using a descriptive analysis method. The results showed that the use of a combination of Urea and TSP fertilizers could affect the protein content of *Spirulina* sp. with the highest protein content obtained in treatment C by 34.33%.

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INTRODUCTION

Spirulina sp. is a microalga of the Cyanophyta group or blue-green algae which has been widely used as natural food in aquaculture activities because it has high nutritional content (Ahmadi *et al.*, 2019). In addition, *Spirulina* sp. could be used as immunostimulants, drugs, cosmetic,s and natural dyes (Richmond, 2004). The nutrients contained in *Spirulina* sp. consist of 60-71%, carbohydrates 16%, fat 8%, Chlorophyll-a 1.6%, Phycocyanin 18%, beta carotene 17%, and 20-30% linoleic acid and vitamins (Suminto, 2009).

Christwardana *et al.* (2013) stated that *Spirulina* sp. contains high protein about 55-70% containing essential amino acids, methionine (1.3-2.75%), cystine (0.5-0.7%), tryptophan (1-1.95%), and lysine (2.6-4.63%). Protein has an

important role in the body, some of which are in the process of forming new cells so that they can repair damaged body tissues. High amino acid levels are good for health because it is one of the ingredients for making protein.

The protein content of *Spirulina* sp. is influenced by environmental conditions such as light intensity, salinity, temperature, and pH. In addition, protein content is also influenced by the length of maintenance and nutritional limitations (especially nitrogen). According to Ulya *et al.* (2018), the biomass quality of *Spirulina* sp. has been shown to deteriorate with the age of culture and nutrients in the media, especially nitrogen. Nitrogen is needed by phytoplankton to support its growth and plays a direct role in the formation of protein in phytoplankton (Kiswati, 2012).

Nitrogen and phosphorus are the two macronutrients that are most needed by microalgae, giving nitrogen and phosphorus at certain concentrations can also increase the protein content of microalgae. (Prayitno, 2016). Both of these nutrients can be obtained from Urea and TSP fertilizers. These types of fertilizers are inorganic (artificial) fertilizers containing nitrogen and phosphorus as the main constituents, Urea fertilizers contain around 45-46% nitrogen, while TSP (Triple Super Phosphate) fertilizers contain around 44-46% phosphorus. Urea and TSP fertilizers can be used as a solution to find other alternative fertilizers as nutrient media, because they are cheap and easy to obtain compared to synthetic media such as walne which are relatively expensive and difficult to obtain.

Currently research on *Spirulina* sp. by using agricultural fertilizers has been done by Sukardi *et al.* (2019), namely by combining 3 types of fertilizers: Urea, ZA and NPK to produce the highest protein 62.37 + 0.01% but the use of only 2 types of fertilizers namely urea and TSP needs to be investigated as an alternative consideration of cost efficiency production.

METHODOLOGY

Place and Time

This research was carried out for 10 days in May 2021 at the Natural Feed Laboratory. Brackish Water Cultivation Fisheries Center (BPBAP) Takalar, South Sulawesi Province.

Research Materials

The tools used in this research are as follows: plastic bucket (10 liters volume), plastic basin (20 liters volume), grinder, sieve, blower, glass stick, aeration faucet and hose, label paper, planktonet filter, 500 liter volume fiber tub, Sedgwick Rafter Counting Cells (SRCC), tissue, microscope, analytical scale, dropper, test tube and measuring cup, 18 watt TL lamp, thermometer, refractometer, pH meter,

filter bag, cover glass, aluminum foil, hand counter, culture rack, submersible pump and camera.

The materials used in this research are: *Spirulina* sp. Urea and TSP Fertilizer, Trace Metal Solution, Vitamin Solution, Seawater, Aquades, Sodium thiosulfate, Chlorine, Chlorine test and Soap.

Research Design

This research is an experimental study, using a completely randomized design (CRD) consisting of 4 treatments and 3 replications. The basis for using Urea and TSP fertilizer doses refers to a previous study by Cahya *et al.* (2020) with the best dose of Urea 0.4 g/l + 0.008 g/l TSP. The treatment given is as follows: A = Urea 0.4 g/l + 0.008 g/l TSP (Control) B = Urea 0.5 g/l + 0.010 g/l TSP C = Urea 0.6 g/l + 0.012 g/l TSP D = Urea 0.7 g/l + 0.014 g/l TSP.

Work Procedure

Preparation of Containers and Sterilization of Culture Instruments

The containers used in this study were 12 plastic buckets with a volume of 10 liters and 4 plastic basins with a volume of 20 liters. The use of plastic containers refers to the research of Muliani *et al.* (2018). Before using the culture containers and equipment, they should be sterilized.

Fertilizer Preparation

Fertilizer preparation was carried out the day before maintenance began, the fertilizer used in this study was Urea and TSP fertilizers combined as growth media for *Spirulina* sp. The fertilizer preparation process is carried out through several stages as follows. Prepare a grinder, Refine the TSP fertilizer first using a grinder and separate the fine and coarse grains using a sieve, then the urea and TSP fertilizers are weighted according to the dosage, then the fertilizer is dissolved with distilled water to 100 ml.

Preparation of Culture Water

The water that will be used in this study is seawater from the main tendon which is filtered using a filter bag into a 500 liter volume reservoir. Furthermore, the sea water is sterilized using chlorine/chlorine with a dose of 30 grams and allowed to stand for 1x24 hours. After that, the sea water is given 15 g of sodium thiosulfate while being given aeration (Suminto, 2009). Then measure the initial salinity with the desired salinity of 30 ppt, because the salinity of the seawater measured at the beginning of the measurement is higher than desired, to determine the salinity so that it needs to be lowered according to the desired salinity, it is calculated using the dilution formula according to Bangun *et al.* (2015).

Then, chlorine test was conducted to find out whether the sea water is neutral or not. The sterile seawater was filled into a basin container for homogenization of each treatment, each filled with 18 liters of water using a measuring cup.

Fertilizer Provision

Fertilizer application is carried out when the sea water is completely neutral from the chlorine content, this fertilizer application is carried out once in a natural feed culture cycle (*Spirulina* sp.). The method of giving fertilizer in this study in the form of a solution (a combination of urea + TSP fertilizer) was carried out by spreading it directly on seawater media. The application of urea and tsp fertilizer solutions in this study was 100 ml/treatment. Then followed by giving a solution of trace metal and vitamin solution each 1 ml/l = 18 ml/treatment, after the fertilizer application stage was given a time lag of 5-10 minutes while aerated to even out the spread of fertilizer.

Spreading Seeds

Initial distribution of *Spirulina* sp. carried out after the homogenization treatment of the culture water until the application of fertilizer was completed in the basin container. Spreading seeds is

done directly and slowly poured into the container. Early seedlings of *Spirulina* sp. used in this study came from the laboratory culture of the Takalar Brackish Water Aquaculture Center (BPBAP), with an initial density of 10,000 cells/ml. To enter the seeds to be cultured, it is calculated using the formula in Prambodo *et al.* (2016).

Maintenance of *Spirulina* sp.

Maintenance period of *Spirulina* sp. carried out for 10 days. During the maintenance process, each container is equipped with aeration and lighting from 18 watt TL lamps for 24 hours.

Parameters

The parameters observed in this study consisted of testing the protein content of the dry weight of *Spirulina* sp. and water quality parameters. *Spirulina* Drying Method is to use direct sunlight for 2-3 days. The protein content test was conducted by measuring protein contained in the dry biomass of *Spirulina* sp. Using the Kjeldahl Method at the end of the study for each treatment. of the proximate analysis test at the end of the research conducted by the laboratory for productivity and water quality FIKP (Faculty of Marine and Fisheries Sciences) Hasanuddin University, Makassar.

Observation of water quality which was measured as a supporting factor for the growth of *Spirulina* sp. carried out every day during the culture implementation process, including temperature, pH, and salinity. Meanwhile, dissolved oxygen (DO) was measured at the beginning and end of maintenance.

Data Analysis

The effect of using a combination of Urea and TSP fertilizers on the Protein Content of *Spirulina* sp. and dry weight was determined using the Mann Whitney U test.

RESULTS AND DISCUSSION

Protein Content

The protein content of *Spirulina* sp. treated with a combination of Urea and TSP fertilizers obtained from the results of the proximate analysis test at the end of

the research conducted by the laboratory for productivity and water quality FIKP Hasanuddin University, Makassar. For more details, the results of the proximate test for protein content can be seen in Figure 1.

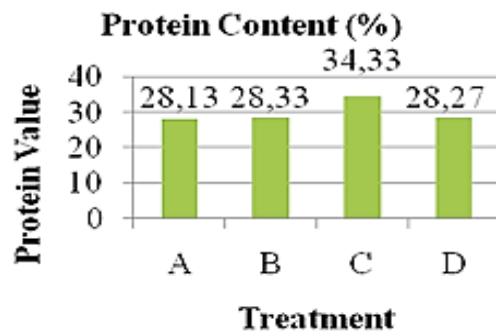


Figure 1. Protein content of *Spirulina* sp.

Based on the graph above, it shows that the protein content of *Spirulina* sp. from the results of cultures that were treated with a combination of Urea and TSP fertilizers, there was a significant difference ($P < 0.05$) with a high value of *Spirulina* sp. protein content found in treatment C (0.5 g/l Urea + 0.012 g/l TSP) as many as 34, 33%, then treatment B (0.6 g/l Urea + 0.010 g/l TSP) was 28.33% and followed by treatment D (0.7 g/l Urea + 0.014 g/l TSP) as much as 28.27%, while the lowest treatment was found in the treatment A (0.4 g/l Urea + 0.008 g/l TSP) as much as 28.13%.

The protein content of *Spirulina* sp. strongly influenced by the nitrate and phosphate elements found in urea and TSP fertilizers which act as constituents of protein compounds in cells. So that the deficiency or excess of these two elements can cause algal cells to experience a decrease in protein content. According to Benavente-Valdés *et al.* (2016), if the availability of nitrogen is limited, it will cause a decrease in protein content. Zhu *et al.* (2015) also stated that amino acid biosynthesis would decrease if nitrogen availability was limited. In addition, according to Ulya *et al.* (2018) that the protein content of microalgae is also influenced by environmental conditions such as temperature, pH, salinity, light

intensity, nutrient limitations (especially nitrogen) and culture age. Based on the results of the study, showed that the protein content of *Spirulina* sp. in each treatment was quite low although between treatments the results were slightly different, this was presumably because the condition of *Spirulina* at the time of harvesting had passed the exponential phase. The research conducted by Jati *et al.* (2012) on the microalgae *Chaetoceros gracilis* stated that the protein content contained harvested in a phase that has passed the exponential phase is lower than the protein content harvested in the exponential phase.

The value of protein content in this study was compared to several previous studies, such as in the study of Ulya *et al.* (2018) using technical fertilizers (Urea, ZA, TSP, FeCl₃, EDTA and the addition of KNO₃ with different doses (50, 100, and 150 ppm). Produced the highest protein content of 66.01% obtained from a dose of 150 ppm KNO₃. Meanwhile in Suminto (2009) which used three different types of fertilizers (Walne, Zarrouk, and TMRL) showed the results of protein content obtained from each fertilizer. of (Walne 67.58%, Zarrouk 66.81%, and TMRL 67.03%). Based on the results of several studies mentioned above, protein content is strongly influenced by the nutritional

content used as a maintenance medium. One of the factors that affect the high yield of protein content in the two previous studies above is the high concentration of nitrogen (nitrate) contained in the media used. Nitrogen is needed in the process of synthesizing amino acids as a constituent of proteins in cells. On the other hand, the lower the nitrogen concentration, the lower the protein content of the cells.

Dry Biomass

Spirulina sp dry biomass obtained from the harvest at the end of the study which was then dried in the sun for 2-3 days, after the sample was dry the next process the sample was taken directly to the productivity and water quality laboratory of FIKP (Faculty of Marine and Fisheries Science) Hasanuddin University, Makassar. For more details, the results can be seen in Figure 2.

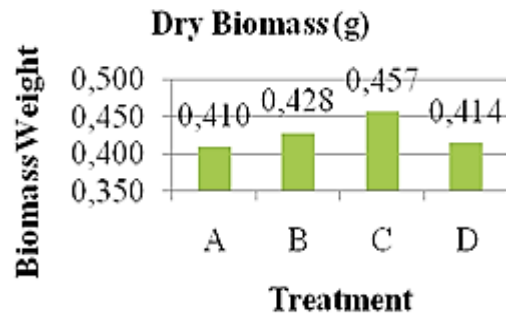


Figure 2. Dried biomass of *Spirulina* sp.

Based on the graph above, it shows that the dry biomass of *Spirulina* sp. the highest was treatment C (0.6 g/l Urea + 0.012 g/l TSP) with a total of 0.457 grams then treatment B (0.5 g/l Urea + 0.010 g/l TSP) with 0.428 and followed by treatment D (0.7 g/l Urea + 0.014 g/l

TSP) of 0.414 grams, while the lowest treatment was found in treatment A (0.4 g/l Urea + 0.008 g/l TSP) of 0.410 grams but there was no significant difference between each treatment ($P > 0,05$). The biomass form of *Spirulina* sp. can be seen in Figure 3.



Figure 3. Clinical sign of shrimp infected by *V. parahaemolyticus*. Description : (A1) sick shrimp, (A2) normal shrimp, (B) empty intestine, (C) the uropod turned red and the gnats.

Spirulina sp. dry biomass weight. influenced by the nitrate and phosphate elements contained in the culture media. The use of Urea and TSP fertilizers in this study is suspected to increase the production of dry biomass of *Spirulina* sp. because the fertilizer provided contains two important nutrients in the growth and

formation of *Spirulina* cells. This is in accordance with Prayitno (2016) that nitrogen and phosphorus are two elements. macroalgae are the most needed macroalgae, the application of nitrogen and phosphorus at certain concentrations can also increase the density of microalgae. According to Mutia *et al.*

(2021), the greater the concentration of nitrate and phosphate added, the greater the amount of biomass produced, but the concentration of nitrate and phosphate that is too high can inhibit growth and increase biomass. According to Hariyati (2008), the amount of biomass produced is directly proportional to the increase in the density of microalgae cells. The weight of the biomass produced will increase if the population density of *Spirulina* sp. increase.

Water Quality

The water quality parameter in this study is one of the supporting factors to meet the needs of *Spirulina* sp. to keep growing. During the study, water quality measurements were carried out every day in the morning between 07.00-08.00 before the daily density calculation was carried out. For more details, the data on the results of water quality measurements can be seen in Table 3.

Table 3. Results of water quality measurement.

Parameters	Measurement results	Optimal Range	Reference
Temperature	26-29	20-30 °C	(Hariyati, 2008)
pH	7.9-8.3	7,2-9,5 ⁽¹⁾ 7-11 ⁽²⁾	¹ (Isnansetyo and Kurniastuty, 1995) ² (Ciferri, 1983)
Salinity	30-33	30-35 ppt	(Isnansetyo and Kurniastuty, 1995)
Dissolved Oxygen	Start: 5.04 End: 6.23	5-7 mg/l	(Fox, 1983; Muliani <i>et al.</i> , 2018)

Based on the results of water quality measurements in the table above, it shows that the temperature obtained during the study ranged from 26-29 °C. temperature is one of the important physical parameters to support the growth of *Spirulina* sp. The temperature obtained during the study was in a relatively normal condition, so that *Spirulina* sp. can adapt and grow well during maintenance. This is in accordance with the opinion of Hariyati (2008) that *Spirulina* sp. can grow well in the temperature range of 20-30 °C. Temperatures that exceed the maximum growth temperature will cause the cessation of cell metabolic processes, because it can inactivate or even turn off many enzymes.

The pH value obtained from the measurement results during the study ranged from 7.9-8.3, the pH condition was still within the normal range to meet the growth of *Spirulina* sp. According to Isnansetyo and Kurniastuty (1995), a good pH for the growth of *Spirulina* sp. ranged from 7.2 to 9.5. However, in some conditions *Spirulina* sp. can still survive up

to pH 11. This is in accordance with the statement of Ciferri (1983) that *Spirulina* sp. can grow well in the pH range of 7-11. Preisig and Andersen (2005) stated that pH can affect the metabolic processes and growth of microalgae as well as affect the physiology of *Spirulina* sp. and can change the availability of nutrients, increasing pH will cause an increase in dissolved CO₂. Bangun *et al.* (2015) also explained that *Spirulina* sp. able to utilize carbon dioxide efficiently even though it is available at very low concentrations, so that *Spirulina* sp. can live well at neutral pH and tolerate more alkaline than acidic conditions.

The salinity values obtained during the study ranged from 30-33 ppt, this range was still in normal conditions for the growth of *Spirulina* sp. This is in accordance with the opinion of Isnansetyo and Kurniastuty (1995) that *Spirulina* sp. can grow well in the salinity range of 30-35 ppt. According to Suminto (2009), the salinity of water can affect the osmotic pressure in a body of water. If the salinity of the waters is high, the osmotic pressure will also be higher, so that the osmotic

pressure is directly related to aquatic organisms in absorbing nutrients for their metabolic processes.

The dissolved oxygen (DO) value obtained from the results of the initial and final measurements during maintenance ranged from 5.04 - 6.23 mg/l. from these results indicate that the range of DO during maintenance can be said to be good and normal to support the life needs of *Spirulina* sp. This is in accordance with the opinion of Muliani *et al.* (2018) that good dissolved oxygen levels and high productivity support the growth of microalgae ranging from 5-7 mg/l, while dissolved oxygen above 7 mg/l is very high. productivity and below 5.0 mg/l less productive. The increase in oxygen content is caused by a large supply of oxygen from photosynthesis and aeration. According to Utomo *et al.* (2020), in some conditions the oxygen supply actually makes agitation/stirring very risky when it is too strong or heavy because it will cause physical damage and inhibit the growth of *Spirulina* sp. then conversely if the oxygen supply is not available or too slow it can also inhibit growth or even cause death in *Spirulina* sp.

CONCLUSION

Based on the results obtained during the study, the authors concluded that the use of a combination of Urea and TSP fertilizers in culture media resulted in the protein content of *Spirulina* sp. the best in treatment C (0.5 g/l Urea + 0.012 g/l TSP) as much as 34.33%.

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