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Does Tofu Wastewater Conversions Nutrient Increase the Content of the *Chlorella pyrenoidosa*?

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ABSTRACT

This study aims to analyze the lipid, carbohydrate and protein content of *Chlorella pyrenoidosa* after being treated with the tofu wastewater. The method used in this study was an experimental method with 4 treatments and 3 replications. The treatment was performed by administering different concentrations of the tofu wastewater to the *C. pyrenoidosa*. The concentrations used were 10%, 15% and 20%. Determination of these concentrations was based on the preliminary test. The main parameters observed were the lipid, carbohydrate and protein content of *C. pyrenoidosa* and the supporting parameters were the growth rate, doubling time and characteristics of the tofu wastewater. The study was conducted for 8 days using the batch culture method. In the exponential phase, the microalgae were harvested and then their contents were analyzed. The data obtained were analyzed using MS Office Excel 2016. The highest content of lipid, carbohydrate and protein of *C. pyrenoidosa* was in the treatment of 20% tofu wastewater, reaching 1.56%; 28.92%; and 28.92%, respectively. Meanwhile, the highest growth rate and the smallest doubling time in the treatment of 20% tofu wastewater accounted for 0.8264 day^{-1} and 0.0349 hours^{-1}. Moreover, the rates of BOD and TSS of the tofu wastewater at the end of the study decreased. It can be concluded that administering different concentrations of the tofu wastewater can affect the lipid, carbohydrate and protein content of *C. pyrenoidosa*. It was suggested that further research is needed to conduct semi-continuous cultivation of *C. pyrenoidosa* using a higher density so that the results obtained can be optimized.

Keywords: carbohydrate, lipid, microalgae, organic wastewater, protein

INTRODUCTION

The tofu industry in Indonesia is a domestic industrial activity carried out by small and medium scale businesses. The tofu industry, during the production process, produces waste in the form of wastewater that comes from soybean washing and tofu clumping (Permatasari et al., 2018). However,

most household-scale tofu industries do not have a wastewater treatment unit, so the waste is often disposed directly into the tributaries. The wastewater contains suspended and organic substances and is a major source of pathogens which, if it is discharged into the water, can reduce the water quality (Vrushali & Kaustav, 2014). The tofu wastewater which is discharged into the tributaries can affect the chemical and physical status of the waste. The tofu wastewater discharged into the tributaries will increase the Total Suspended Solid (TSS), thus increasing turbidity and causing high oxygen consumption of the bacteria during the degradation, so that the Biological Oxygen Demand (BOD) is high. In addition, it can cause water to be turbid and dirty (Naidoo & Olaniran, 2014).

The organic compounds in the tofu wastewater are 40–60% protein, 25–50% carbohydrates and 10% fat. The organic compounds contained in the tofu wastewater can be broken down into macronutrients with the help of decomposing bacteria, which are then utilized by microalgae (Harahap et al., 2013). One type of microalgae that is commonly found in tributaries is the *C. pyrenoidosa*. *Chlorella pyrenoidosa* is a species of unicellular freshwater green algae. The cell is self-contained with a spherical shape with a diameter of 3–8 microns, and has cup-shaped chloroplasts as well as hard cell walls (Nicoletti, 2016).

The *C. pyrenoidosa* contain 57% proteins, 26% carbohydrates and 2% lipids (Singh et al., 2011). The high protein content of *C. pyrenoidosa* can be used as a natural feed ingredient for aquatic organisms (Bolgovics et al., 2019.), while the carbohydrate content can be used as an ingredient in the manufacturing of bioethanol (Assadad et al., 2010), and the lipid content has a great potential as a substitute for diesel fuel (Kim, 2015.).

The tofu wastewater can be utilized by converting it as nutrient needs for microorganisms. The organic compounds contained in the tofu wastewater can be degraded into macronutrients needed by autotrophic organisms, such as microalgae (Putri et al., 2018). The *C. pyrenoidosa* requires a source of nutrients in the form of N and P in their growth process. The tofu wastewater contains abundant N and P which was previously decomposed by bacteria then can be used by microalgae as a source of nutrition to grow and produce carbohydrates, proteins, and lipids.

On the basis of the description above, the tofu wastewater can be used as additional nutrients in the *C. pyrenoidosa* culture. This study aimed to analyze the effects of giving the tofu wastewater at different concentrations on the carbohydrate, protein and lipid content of the *C. pyrenoidosa*. Furthermore, this study also measures the supporting parameters, including the growth rate, doubling time and characteristics of the tofu wastewater before and after treatment.

MATERIALS AND METHODS

The microalgae used in this study were *C. pyrenoidosa*. The microalgae seeds were obtained from CV. Ugo Plankton, Central Java. The microalgae were cultivated in 2.5 L of sterile fresh water medium and given 2.5 mL of Walne nutrient, 2.5 mL of vitamin and 250 mL of microalgae strains. The microalgae were cultivated for 4–5 days before being used for the research.

Preliminary Test

The determination of the tofu wastewater concentration was based on the results of a preliminary test. The tofu wastewater was obtained from the Tofu Factory in Sukun area, Malang, East Java. The preliminary test used various concentrations of tofu wastewater amounting to 10%, 15%, 20%, 25%, 30% and 40% of the medium volume to determine the concentration of the tofu wastewater best used for the growth of *C. pyrenoidosa*. The results of the preliminary test indicated that *C. pyrenoidosa* can grow well at the tofu wastewater concentrations of 10%, 15% and 20%. Therefore, the tofu wastewater concentrations of 10%, 15% and 20% of the volume of culture medium were used in this present study. The determination of the volume of wastewater used was based on the following formula:

$$\text{Tofu wastewater} = \frac{\text{The desired conc. (\%)}}{100} \text{ x total medium volume} \quad (1)$$

$$\text{Water volume used} = \text{Total medium vol.} - \text{tofu wastewater vol.} \quad (2)$$

Preparation of the tofu wastewater medium was as follows:

- Concentration of 10% = 500 mL of tofu wastewater + 4500 mL of fresh water;
- Concentration of 15% = 750 mL of tofu wastewater + 4250 mL of fresh water;
- Concentration of 20% = 1000 mL of tofu wastewater + 4000 mL of fresh water.

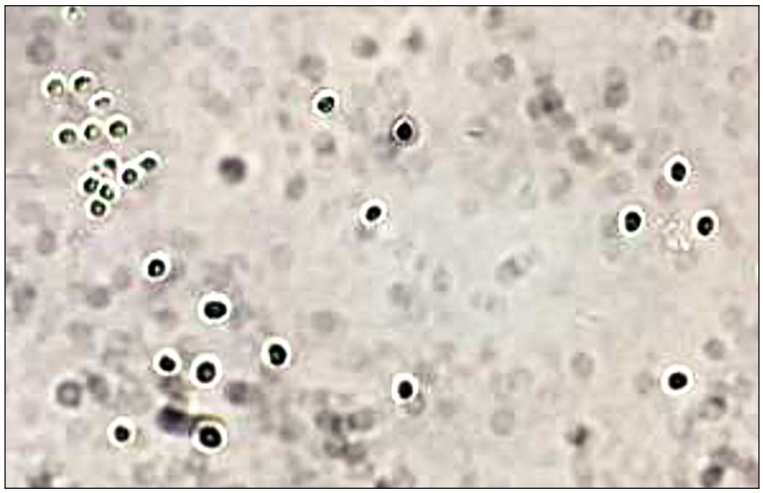

Figure 1. *Chlorella pyrenoidosa* (Research Documentation, 2020)

Experimental Design

The research was conducted at the Hydrobiology Laboratory, Hydrobiology Division, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang in February-March 2020. The study used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment applied was the addition of the tofu wastewater in the concentration of 10%, 15% and 20% of the volume of culture medium. Determination of the tofu wastewater concentration was based on the preliminary test that had been conducted.

Analyses of Microalga Content

The analyses of the carbohydrate content used the Luff Schoorl method. The procedure involved taking 0.1 gram of *C. pyrenoidosa* microalgae and placing them into a 250 mL Erlenmeyer, and then 50 mL of distilled water and 5 mL of 25% HCl were added. The sample solution was then heated at 100°C for 3 hours, and subsequently cooled down to room temperature. The sample solution was then neutralized with 25% of NaOH to pH 7, before being transferred into a 100 mL measuring flask and filtered using a filter paper. A sample of 25 mL was added to 25 mL of Luff Schoorl solution in the Erlenmeyer. The blank solution was made with 25 mL of Luff Schoorl solution added to 25 mL of distilled water, and then the solution was boiled for 10 minutes, before being cooled down later on. The solution was added with 15 mL of 20% KI and 25 mL of 25% H_2SO_4, and then it was covered and placed in a dark place for 30 minutes. The solution was titrated using 0.1 N $Na_2S_2O_3$ solution using a starch indicator as much as 2–3 mL. The difference between the blank titration and the sample titration was multiplied by 0.9 to obtain the starch content value. The yield of carbohydrates was calculated using the formula (Ifmaily, 2018):

$$Carbohydrates\ yield = \frac{mg\ glucose\ x\ FP\ x\ 0{,}9}{mg\ sample} 100\% \quad (3)$$

where: *FP* – mL of filtrate titration.

The protein analyses were performed using the Kjeldahl method. The protein analyses were carried out in 3 processes, namely the process of digestion, distillation and titration. The harvested sample was placed into a Kjeldahl flask and was added 10 ml of sulfuric acid and 5 grams of catalyst. The sample was subjected to a digestion process until the liquid was clear green. The results of the digestion were diluted with distilled water to 100 ml, and then 10 ml was taken to be placed into a distillation flask. Following this, 10 ml of 30% NaOH solution was added. The distillation process was carried out for 20 minutes, and then the results of the distillation process were stored in an Erlenmeyer containing 25 ml of 0.1 N HCl solution. The results of the distillation were titrated with 0.1 N NaOH solution. The final point of the titration was marked in pink. The protein analyses was performed using the formula (Rosaini et al., H., 2015):

$$\text{Protein content (\%)} = \text{Nitrogen x } 6.25 \quad (4)$$

The lipid analyses used the Soxhlet method in which *C. pyrenoidosa* were harvested and dried. The dry samples were extracted in a Soxhlet tool

with petroleum ether solvent for ± 6 hours. After extraction, a vacuum evaporator was used to vaporize the n-hexane. The lipid yield was then weighed and calculated using the formula (Boni et al., 2018):

$$\% \text{ Yield} = \frac{\text{Extracted lipid weight}}{\text{Sample weight}} \text{x } 100\% \quad (5)$$

The analyses were carried out at the Nutrition Laboratory, Department of Health Nutrition, Faculty of Public Health, Airlangga University.

Supporting Parameters

During the study, the growth rate (μ) and doubling time (G) of *C. pyrenoidosa* were calculated. The growth rate was calculated during the cultivation period until the exponential phase, which was on day 8. The growth rate was calculated using the following formula (Arsad et al., 2019):

$$x = \frac{\text{Ln N2} - \text{Ln N1}}{\text{d2} - \text{d1}} \quad (6)$$

where: x – growth rate,
$N1$ – initial cell density,
$N2$ – cell density on day-t,
$d1$ – initial culture time,
$d2$ – final culture time.

Meanwhile, the doubling time can be calculated using the formula (Prayitno, 2016):

$$G = \frac{\ln 2}{24\ (x)} \quad (6)$$

where: G – doubling time; x – growth rate.

In addition, the values of BOD (*Biochemical Oxygen Demand*) and TSS (*Total Suspended Solid*) were measured to determine the initial and final characteristics of the tofu wastewater. The measurement of BOD used the APHA 5210 B-2017 (APHA, 2017) method and the measurement of TSS used the APHA 2540 D-2017 (APHA, 2017) method, which were carried out at the Environmental Laboratory of PJT 1 Malang.

Statistical Analyses

The data of the carbohydrate, protein and lipid content of *C. pyrenoidosa* were statistically processed using the method of analysis of variance (ANOVA) with a completely randomized design at a significant level of 5%. If it was is significantly different, then the LSD test (Least Significant Difference) was carried out to find out which treatment was significant or had a significant effect. The data obtained were analyzed using MS Office Excel 2016.

RESULTS AND DISCUSSIONS

Carbohydrate, Protein and Lipid Content

On the basis of the results of the homogeneity test (Table 1), the carbohydrate content of *C. pyrenoidosa* is homogeneous with the test results showing the sig. value of 0.177. The ANOVA test results show the value of F count (40187.37)> F table 5% (4.07), which means that administering the tofu wastewater with different concentrations has a significant effect on the *C. pyrenoidosa* carbohydrates. Furthermore, the Least Significant Difference Test (LSD) was carried out. On the basis of the LSD test results, it was found that 20% tofu wastewater concentration is a treatment that has a significant effect on the carbohydrate content of this microalgae. The highest value of carbohydrate content of *C. pyrenoidosa* is in the treatment of the tofu wastewater with a concentration of 20% at 28.92% and the lowest is obtained in the control treatment (without wastewater) at 23.61%. The difference in the concentration of the tofu wastewater indicates that the carbohydrate content of *C. pyrenoidosa* increases. The difference in carbohydrate levels in each treatment is due to the differences in the levels of nutrients contained. The level of glucose contained in the tofu wastewater is used by *C. pyrenoidosa* for growth. The more glucose levels that are used by microalgae, the higher the carbohydrate content (Yudhistira et al., 2016). However, if the concentration of the tofu wastewater is too high, it can also lead to the *C. pyrenoidosa* growth due to an increase in the concentration of the medium, which can inhibit light entering the medium (Larasati et al., 2019).

The protein content of *C. pyrenoiodosa* is homogeneous with the test results showing the sig. value of 0.051. The ANOVA test results show the value of F test (517.933)> F table 5% (5.14), which means that the provision of different tofu wastewater concentrations has a significant effect on the protein of this species. Furthermore, the Least Significant Difference Test (LSD) was carried out. On the basis of the results of the LSD test, it was found that 20% tofu wastewater concentration is a treatment that had a significant effect on the protein content of *C. pyrenoidosa*. The highest protein

Table 1. Carbohydrate, Protein and Lipids Content of *C. Pyrenoidosa.*

Treatment	Carbohydrate (%)	Protein (%)	Lipid (%)
Control	23.61±0,04	54.66±0,1	1.59±0.03
A (10%)	26.73±0.01	56.40±0,2	1.2±0.02
B (15%)	27.83±0,01	57.99±0,03	1.78±0.01
C (15%)	28.92±0.01	58.61±0,04	1.86±0.01

value is obtained in the treatment of the tofu wastewater with a concentration of 20% at 58.61% and the lowest is obtained in the control treatment at 56.40%. This happens because the treatment of the tofu wastewater with a concentration of 20% yields more nutrients when compared to other treatments. The high protein content in the tofu wastewater treatment with a concentration of 20% indicates that the protein synthesis process in the microalgae body which utilizes nutrients, namely nitrogen, runs optimally. The nitrogen element contained in the tofu wastewater is a major component in the formation of chlorophyll and the formation of protein in *C. pyrenoidosa*. Nitrogen in the form of nitrate (NO_3) and ammonium ($NH4^+$) in the culture medium is utilized by *C. pyrenoidosa* for the purposes of cell metabolism such as catabolism and assimilation, especially protein biosynthesis. Nitrogen which is used in the amino acid biosynthesis process is sufficient so that the protein value is high enough. The high nitrogen source in the culture medium can also increase the cell biomass and protein content of microalgae during cultivation (Negi et al., 2016). The protein content in the body of *C. pyrenoidosa* plays a role in the process of forming new cells so that it can repair damaged body tissue. In addition, the high protein content of *C. pyrenoidosa* in this study can be used as a natural food ingredient for aquatic organisms such as fish, crustaceans and bivalves (Widianingsih et al., 2018).

The lipid content of *C. pyrenoidosa* is homogeneous with the test results showing the sig. value of 0. 494 ($p > 0.05$). The ANOVA test results show the value of F count (112.67)> F table 5% (4.07), which means that the provision of different concentrations of tofu wastewater affects the lipid content of the *C. pyrenoidosa* microalgae. Furthermore, the Least Significant Difference (LSD) test was carried out to determine which treatment had the most effect on the lipid content of *C. pyrenoidosa*. On the basis of the results of the LSD test, it was found that 20% tofu wastewater concentration is a treatment that has a significant effect on a lipid content of 1.86%. The lipid content increases along with the tofu wastewater concentration. This occurs as the increase in the tofu wastewater concentration is proportional to the carbon content in the cultivation medium. Higher ratios of carbon and nitrogen result in higher lipids of microalgae (Widayat et al., 2018). The carbon element is absorbed by microalgae, supported by oxygen. Elemental carbon will be converted into acetyl CoA in the citric acid cycle, and then Acetyl CoA forms saturated fatty acids in the lipogenesis process. The saturated fatty acids formed will react with glycerol from the glycolysis process and form Triacylglycerol which will increase the lipid content of microalgae (Salim, 2013).

Growth Rate and Doubling Time

The highest growth rate is in the tofu wastewater concentration of 20% at 0.8264 $days^{-1}$ and the lowest is in the control group at 0.7471 $days^{-1}$. The growth rate is a parameter that describes the rate of growth of microalgae cells per unit time. The higher the concentration of tofu wastewater addition, the greater the value of its growth rate. This is due to the presence of nutrients in the tofu wastewater such as nitrogen and phosphate, which are used by *C. pyrenoidosa* to support its growth. Nitrogen and phosphorus are macronutrients needed for the growth of this microalgae. The availability of nitrogen and phosphorus in the culture medium affects the growth of microalgae. The growth and productivity of the *Chlorella* biomass will decrease when the nitrogen and phosphorus concentrations are reduced (Khan et al., 2018).

The doubling time obtains a value of 0.0387 $hours^{-1}$ in the control treatment, of 0.0359 $hours^{-1}$ in the tofu wastewater concentration of 10%, of 0.0353 $hours^{-1}$ in the tofu wastewater concentration of 15% and of 0.0349 $hours^{-1}$ in the tofu wastewater concentration of 20%. The lowest doubling time is the shortest time required for cell division or cell multiplication (Sigalingging et al., 2019). The growth rate will be directly proportional to the growth of the *Chlorella pyrenoidosa* cells, while the higher the growth rate is, the smaller the doubling time is, and vice versa (Rosenberg et al., 2014).

Characteristics of the Tofu Wastewater

The measurement of the tofu wastewater parameters was carried out before the treatment of

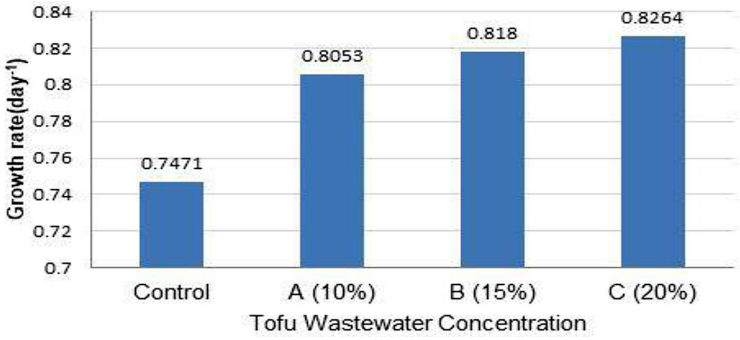

Figure 2. Growth rate of *Chlorella pyrenoidosa*

C. pyrenoidosa to determine the initial characteristics of the tofu wastewater. The measurement results can be seen in Table 2. It is known that the BOD_5 and TSS parameters exceed the quality standards, which are 1325 mg. L^{-1} and 466 mg. L^{-1}respectively. The high BOD value in the tofu wastewater is due to the high protein content, which is an organic substance in tofu wastewater, so it requires dissolved oxygen for the decomposition process, while the high TSS value in the tofu wastewater comes from the remaining solid soybean which has not been completely filtered because it still uses simple technology (Ahmad, & Adiningsih, 2019). The measurement of waste parameters at the concentration of 10%, 15%, and 20% was carried out at the beginning and end of cultivation to see the decrease in the BOD_5 and TSS concentrations, caused by *C. pyrenoidosa*. The measurement results can be seen in Table 3.

The Table 3 shows a decrease in the BOD_5 and TSS concentrations at the end of cultivation. The decrease in the BOD_5 concentration occurs due to the presence of the *C. pyrenoidosa* which absorb the organic compounds in the tofu wastewater as nutrients. The BOD value at the beginning of the study exceeds the established quality standard and at the end of the study, after being given *C. pyrenoidosa,* it decreases to below the quality standard. BOD is the amount of oxygen used by *Chlorella* to oxidize the organic substances contained in the tofu wastewater. The BOD content, if untreated, will be higher and can reduce the oxygen in the water (Simatupang et al., 2017). The decrease in the BOD levels in the tofu wastewater occurs because *C. pyrenoidosa* absorbs the organic compounds contained as nutrients for *C. pyrenoidosa.* This utilization will reduce the concentration of BOD in the tofu wastewater (Singh & Dhar, 2010). The 20% concentration treatment causes a slight decrease in TSS. This is because the greater the concentration of tofu wastewater added, the more suspended materials will be in the medium. The suspended materials in the tofu wastewater that cannot be absorbed by microalgae will settle at the bottom and cause a high TSS concentration (Munawaroh et al., 2013).

Table 2. Initial characteristics of tofu wastewater

Parameter	Units	Analysis results	Standard*
BOD_5	$mg{\cdot}L^{-1}$	1325	150
TSS	$mg{\cdot}L^{-1}$	466	400

* KepMenLH no 51 Year 1995 on the Liquid Waste Quality Standard for Industrial Activities

Table 3. The results of measurement of tofu wastewater after treatment

Treatment	BOD_5 ($mg{\cdot}L^{-1}$)		TSS ($mg{\cdot}L^{-1}$)	
	Initial	Final	Initial	Final
10%	298	120	50.9	9.8
15%	453	145	46.9	19.1
20%	761	149	64.5	49.9

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