Utilization of tofu wastewater as *Chlorella pyrenoidosa* growth medium

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Abstract. This study aims to analyze the growth of microalgae *Chlorella pyrenoidosa* cultured in the medium from a tofu wastewater area treatment. The study used an experimental method with 4 treatments and 3 replications. The treatments were carried out by administering different concentrations of tofu wastewater to *C. pyrenoidosa*. The concentrations used were 10%, 15% and 20% with a light intensity of 5000 lux. The main parameter observed was microalgae growth, while the supporting parameter was water quality, which affected the growth of *C. pyrenoidosa*. The results indicated that different tofu wastewater treatments produced different densities of *C. pyrenoidosa*. The highest density of *C. pyrenoidosa* was found in the tofu wastewater treatment of 20% ($3.253x10^4$ cells mL^{-1}). The ranges of temperature, pH and dissolved oxygen from the culture were 27.4-28.5°C, 7.1-8.76, and 6.9-8 mg L^{-1}, respectively. The analysis of variance (ANOVA) test revealed that different concentrations of tofu wastewater can affect the density of *C. pyrenoidosa*. The most significant differences were observed in the concentration of 20%. Moreover, the present study suggests further research on conducting semi-continuous cultures of *C. pyrenoidosa* with tofu wastewater treatment.
Key Words: liquid waste, microalgae, nutrient, water quality.

Introduction. The tofu industry is one of the most developed domestic industrial activities in Indonesia, which are dominated by small and medium scale businesses. Therefore, most of these businesses do not have wastewater treatment plants to treat their waste. In the production process, the tofu industry generates two types of waste, liquid and solid. This liquid waste comes from soybean washing in form of wastewater, whereas the solid waste comes from tofu production in the form of tofu clumps. The resulting wastewater is often discharged directly into rivers or tributaries without any treatment. Thus, the wastewater causes pollution due to its high organic content that may have negative impacts on the environment (Azmi et al 2016).

Wastewater from tofu production contains considerable amounts of organic substances such as proteins, carbohydrates and fats, usually in the following percentages: 40-60%, 25-50%, and 10%, respectively (Dianursantia et al 2014). Organic substances contained in tofu wastewater, if decomposed, will produce gases, such as nitrogen, oxygen, hydrogen sulfide, ammonia, carbon dioxide and methane. If tofu wastewater is discharged directly into the environment, there will be organic deposition of substances in the water bodies, decomposition and the growth of pathogenic microorganisms (Widayat et al 2018).

An effort should be made to reduce the direct discharge of tofu wastewater into rivers or tributaries, to minimize its impact on the environment. The utilization of this wastewater for microalgae could be a good option, because microalgae can utilize the nutrients from tofu wastewater. According to previous studies, many microalgae species such as *Scenedesmus* sp. are alternatives in wastewater treatment, growing well in wastewater and reducing the heavy metal content in wastewater (Ballen-Segura et al 2016). *Chlorella vulgaris* is able to promote a decrease of the biological oxygen demand (BOD) and chemical oxygen demand (COD) values from waste, this being associated with an increase in the growth rate of algae (Mohammed et al 2016). *C. vulgaris* grows well in autotrophic and mixotrophic conditions using wastewater, and can remove nitrogen and phosphorus in wastewater (Ge et al 2018). *Chlamydomonas* can grow in wastewater with suitable CO_2 levels (Kong et al 2010). *Haematococcus* can grow well in wastewater and remove nitrogen and phosphorus (Kang et al 2006). *Spirulina* can accumulate pollutants such as organic and inorganic compounds in wastewater so that wastewater can be used as growth media (Dolatabadi & Hosseini 2016). Microalgae *Chlorella pyrenoidosa* could be one of the alternatives since this species lives in fresh water (Husma 2017). *C. pyrenoidosa* is a unicellular microalga with a diameter of 4 to 5 μm, reproduces asexually and has a cup-shaped chloroplast with thick cell walls (Murray et al 2016).

The utilization of tofu wastewater is done by converting it as nutrient for microorganisms, in this case microalgae. Microalgae can utilize organic compounds contained in the tofu wastewater to increase biomass and microalgae growth. Organic compounds contained in tofu wastewater can be broken down by microalgae into macronutrients (Putri et al 2018). On the other hand, in the process of growth, *C. pyrenoidosa* requires a source of nutrients in the form of N and P. Nutritional sources in the form of N and P are often found in tofu wastewater. In other words, tofu wastewater contains abundant organic compounds and nutrients in the form of N and P, which can be utilized by microalgae (Arsad et al 2019).

Based on the description above, tofu wastewater can be used as an additional source of nutrients for microalgae. Therefore, research on the effects of tofu wastewater concentration on the growth of *C. pyrenoidosa* need to be conducted. This study aims to analyze the effects of tofu wastewater at different concentrations on the growth of *C. pyrenoidosa*. Furthermore, this study also presents the water quality parameters that support the growth of *C. pyrenoidosa*.

Material and Method

Microalgae preparation. The microalgae species used in this research were *C. pyrenoidosa* (Figure 1). This strain was obtained from CV Ugo Plankton, Purworejo Regency, Central Java. The *C. pyrenoidosa* cultures were reared in 2.5 L of freshwater which had been sterilized, with an addition of 2.5 mL of Walne fertilizer, 2.5 mL of vitamins and 250 mL of microalgae based on Balai Budidaya Air Payau Situbondo composition media. Microalgae were cultivated for 5 days in the laboratory of Hydrobiology, Faculty of Fisheries and Marine Science Brawijaya University, Malang, in 2020, before the experiment.

Tofu wastewater concentration. Tofu wastewater was obtained from the tofu factory in Sukun area, Malang, East Java. Tofu wastewater used comes from the process of deposition of tofu, which leaves water as a result of the tofu deposit. The determination of the concentration of tofu wastewater was based on the preliminary study that had been done.

In the preliminary study, the research treatments were 10%, 15%, 20%, 25%, 30% and 40%. This preliminary study aimed to determine the best concentrations of tofu wastewater for the growth of *C. pyrenoidosa*. Then, the preliminary research results showed that the best concentrations of tofu wastewater were 10%, 15% and 20%, which later were used as a reference to carry out the main research. In the main research, the preparation of tofu wastewater medium was based on the concentration treatments, which were 10%, 15% and 20%, and 5000 mL were needed for each treatment.

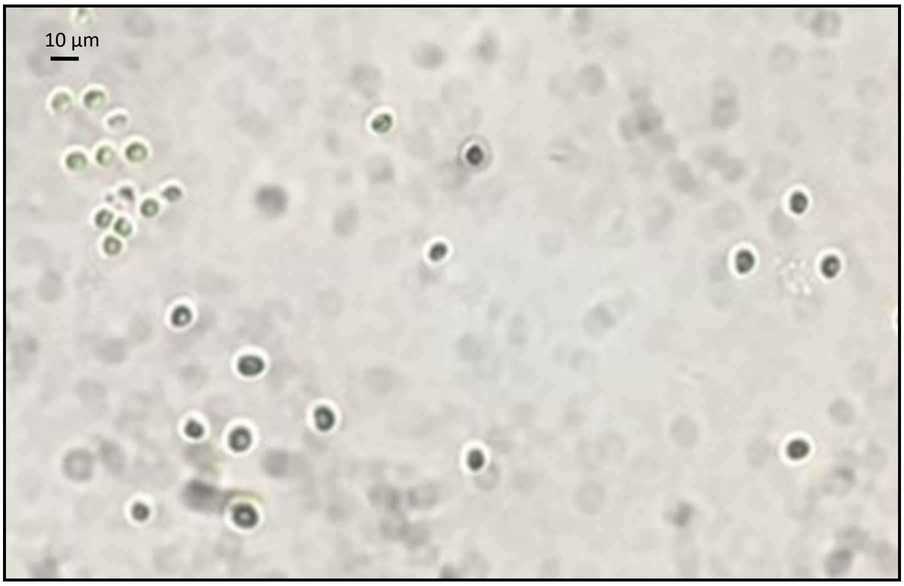

Figure 1. *Chlorella pyrenoidosa* under the light microscope (x40 magnification).

Tofu wastewater = [the desired concentration (%)/100] x total medium volume

Water volume used = total medium volume - tofu wastewater volume

The preparation of tofu wastewater was as follows:
1. Concentration of 10% (A) = 500 mL of tofu wastewater + 4500 mL of fresh water
2. Concentration of 15% (B) = 750 mL of tofu wastewater + 4250 mL of fresh water
3. Concentration of 20% (C) = 1000 mL of tofu wastewater + 4000 mL of fresh water

Experiment setup. Water quality for *C. pyrenoidosa* culture at the beginning of the study was set at optimum conditions. Therefore, *C. pyrenoidosa* could grow optimally (Figure 2).

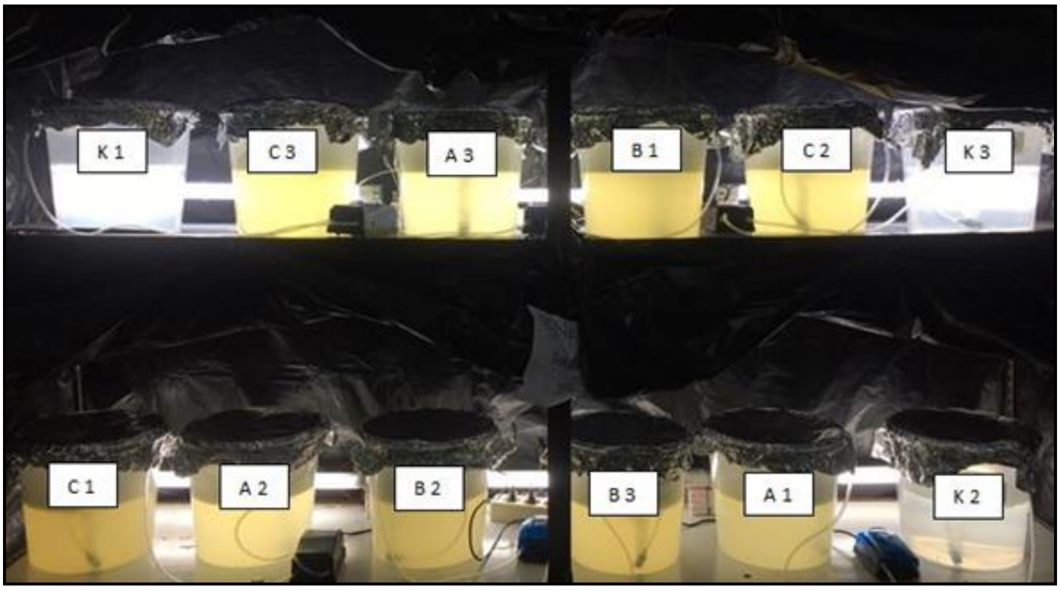

Figure 2. Culture setup of *Chlorella pyrenoidosa*. A - treatment with 10% tofu wastewater; B - treatment with 15% tofu wastewater; C - treatment with 20% tofu wastewater; K - control with 0% tofu wastewater; 1, 2, 3 - replications.

Density of microalgae. The initial microalgae density used in this study was 100000 cells mL^{-1}. Microalgae growth was observed every day for 7 days. Meanwhile, the calculation of microalgae density was conducted using a haemocytometer (Neubauer chamber) with the formula mentioned by Padang et al (2018):

Density = N x 10^4 cells mL^{-1}

Where: N - number of cells obtained; 10^4 - haemocytometer constant.

Measurement of water quality parameters. Measurement of temperature and dissolved oxygen (DO) was carried out using an oxygen meter (Lutron DO-5510). The pH measurement was conducted with a pH meter (C232 pH meter pH-2). The light intensity was determined using a lux meter (LX-1010B). The photoperiod was set 14:10 hours (light:dark) daily during the experiment. Water quality parameter measurements were carried out every day during the trial period.

Data analysis. Data from observations of *C. pyrenoidosa* density were processed statistically using analysis of variance (ANOVA) method with a completely randomized design at 5% significance level where the normality and homogeneity were tested before. If it was significantly different, then the least significant difference (LSD) test was conducted to find out which treatment was significant or had a significant effect. The data obtained were analyzed using Microsoft Office Excel 2016.

Results and Discussion. Based on the observation of *C. pyrenoidosa* treated with tofu wastewater, the density obtained is presented in Figure 3.

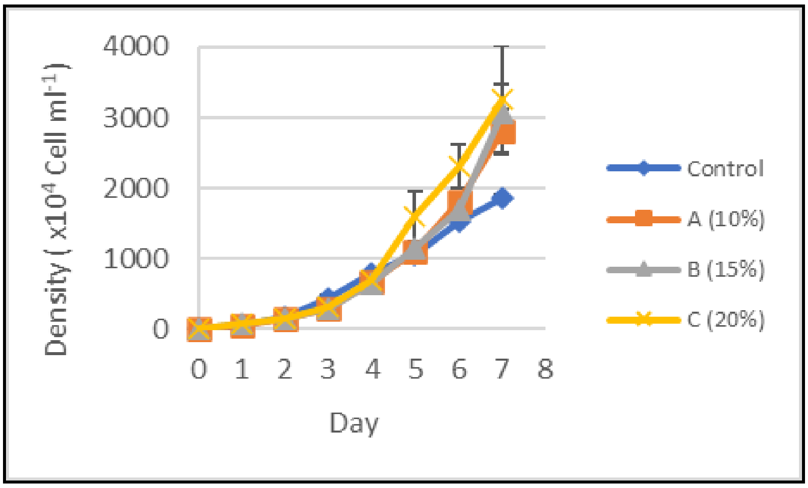

Figure 3. The growth of *Chlorella pyrenoidosa*.

Supporting parameters of microalgae growth. The temperature in the treatments is presented in Figure 4. The temperature ranged from 27.4 to 28.5°C. This range was still in the optimal temperature category for the growth of *C. pyrenoidosa*. This is in accordance with the statement of Singh & Singh (2015), that *C. pyrenoidosa* cultivated in the medium had an optimal growth in temperatures between 25-30°C. The temperature of the liquid waste from soybean stew of the tofu industry reaches 80°C. The waste input with high temperatures will affect the temperature of the water. Water temperature will increase and can disturb aquatic organisms (Mardhia & Abdullah 2018).

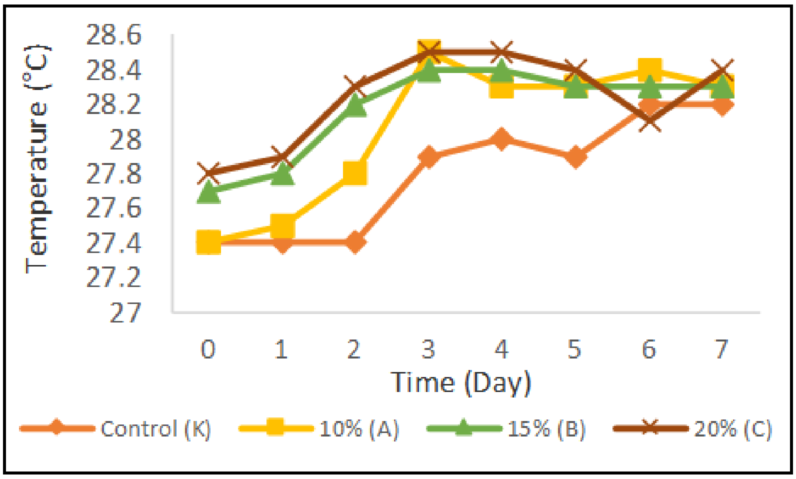

Figure 4. Temperature of the growth medium for *Chlorella pyrenoidosa*.

The observed pH during the study (Figure 5) ranged from 7.1 to 8.76, being in the optimal range. This is in line with the statement of Han et al (2013) that the optimal pH for the growth of *C. pyrenoidosa* is between 7-9. Moreover, the results of pH in each treatment tend to increase. The changes in pH values in the culture medium were assumed to be the result of microalgal photosynthetic activity. The use of CO_2 and HCO_3^- as a source of carbon in photosynthesis would leave OH^- in microalgae cells. This would be neutralized by absorbing H^+ from the extracellular environment. Ultimately, the reduction of H^+ in the culture medium will lead to an increase in pH (Chi et al 2011). Usually, the pH of tofu industry waste has values that do not meet quality standards, being acidic. Wastewater from tofu industry activities discharged into waters has the potential to change the pH of water and can disrupt the life of aquatic organisms (Mardhia & Abdullah 2018).

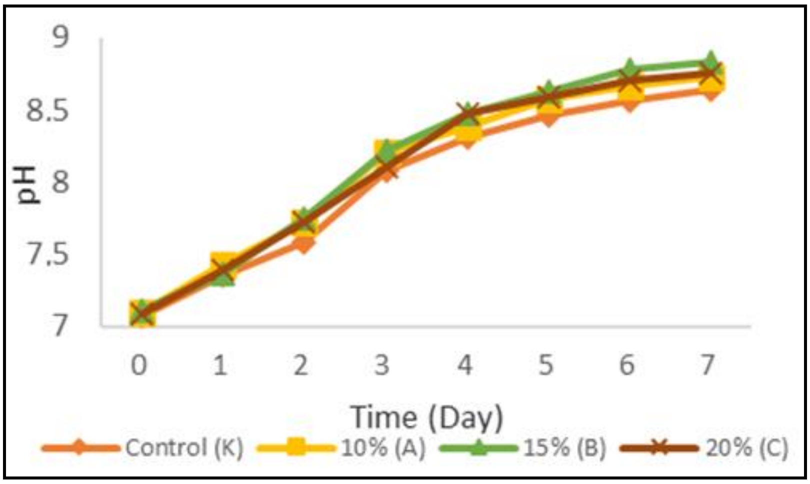

Figure 5. pH of the medium of *Chlorella pyrenoidosa*.

DO values in the *C. pyrenoidosa* culture medium during the study were between 6.9-8 mg L^{-1} (Figure 6). The DO values were in the optimal range for the growth of *C. pyrenoidosa*. According to Mufidah et al (2017), the values of DO for microalgae growth are between 4-8 mg L^{-1}. The DO in the culture medium was obtained from the photosynthesis of microalgae. Decreased levels of DO in the culture medium could occur due to the decomposition process caused by the large number of microalgae experiencing a death phase. Cheng et al (2019) state that the decrease in DO is caused by dead microalgae, resulting in competition in the use of oxygen. The amount of organic substances contained in tofu liquid waste can also cause a decrease in DO levels. With more organic matter in the water, less oxygen content is dissolved in it. The low value of DO in water adversely affects the life of aquatic organisms (Sepriani et al 2016).

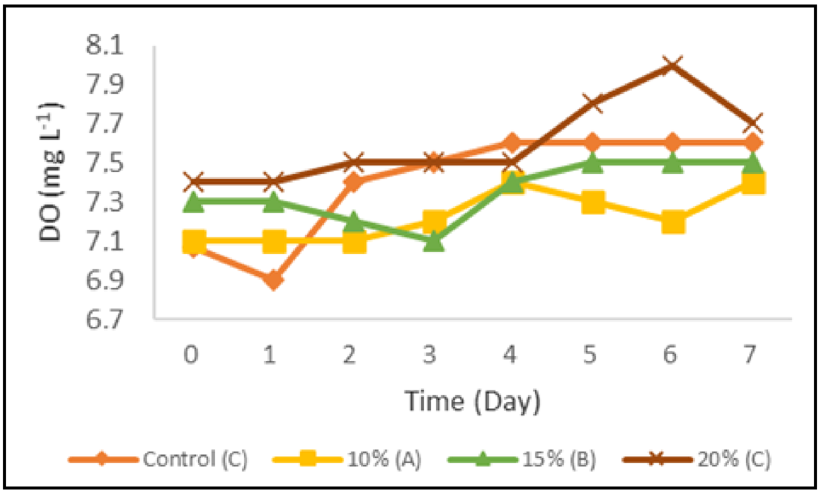

Figure 6. Dissolved oxygen in the medium of *Chlorella pyrenoidosa*.

Based on the normality and homogeneity tests on *C. pyrenoidosa*, cell density data was normal and homogeneous, with the results of the normality test showing a sig value of 0.43 ($p>0.05$) and the homogeneity test showing a value of sig of 0.058 ($p>0.05$). The ANOVA test showed that the concentration of tofu wastewater significantly affected the growth of *C. pyrenoidosa* ($p<0.05$). Furthermore, the LSD test was performed to determine which treatment affected most the growth of *C. pyrenoidosa*. The LSD demonstrated that tofu wastewater concentration of 20% had a significant effect. This was due to the fact that tofu wastewater concentration of 20% had higher levels of nutrients, so *C. pyrenoidosa* could utilize more nutrients for growth. The statistical analyses showed that a higher concentration produce better growth based on the algal cell density. The tofu wastewater was converted to inorganic N and P compounds by bacteria decomposition and further used by the microalgae.

The average density of *C. pyrenoidosa* in all treatments experienced a lag phase on day 1 and an exponential phase from day 2 to day 7. This exponential phase began with an increase in cell density. At the beginning of cultivation, the average density of the control was relatively higher than that of the tofu wastewater treatments of 10%, 15% and 20%. This was influenced by nutrients in the tofu wastewater medium. If the nutrients in the waste were too abundant, the reduction process of organic compounds and microalgae growth was hampered because microalgae required longer time to adapt (Munir et al 2017).

C. pyrenoidosa experienced an exponential peak on day 7. The highest exponential peak density value was produced in the 20% tofu wastewater treatment medium, with 3253×10^4 cells mL^{-1} and the lowest was in the control, with 1867×10^4 cells mL^{-1}. The high density of *C. pyrenoidosa* found in the tofu wastewater treatment of 20% was due to the higher number of nutrients in the medium. Nutrients from tofu wastewater are used by *C. pyrenoidosa* to support its growth. In accordance with the statement of Paes et al (2016), *C. pyrenoidosa* microalgae consume the available nutrients in the medium, so microalgae can grow optimally. If nutrients and environment are maintained in optimum conditions, theoretically, the exponential phase will continue.

Moreover, light intensity is a major factor in the growth of microalgae because in the presence of light, microalgae can carry out photosynthesis. Light is needed by microalgae as an energy source. In addition, the growth of microalgae is also influenced by the presence of light and dark periods. If the period of light is lacking, the growth rate will be low, and vice versa (Afriani et al 2018). According to Safitri et al (2013), the intensity of light and photoperiod are related because they play a role in the process of photosynthesis as an energy source for cell metabolism.

Conclusions. The utilization of tofu wastewater as a culture medium can increase the growth of *C. pyrenoidosa*. The best growth of *C. pyrenoidosa* was in the treatment with tofu wastewater concentration of 20% with a density of 3253×10^4 cells mL^{-1}. The values of temperature, pH and DO were in the optimum range, 27.4-28.5°C, 7.1-8.76 and 6.9-8 mg L^{-1}, respectively. In the future, a comprehensive study using semi-continuous culture of *C. pyrenoidosa* with tofu wastewater treatment merits further investigation.

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References

Afriani S., Uju, Setyaningsih I., 2018 [Chemical composition of *Spirulina platensis* cultivated in photobioreactor with different photoperiod]. Jurnal Pengolahan Hasil Perikanan Indonesia 21(3):471-479. [In Indonesian].

Arsad S., Stavrakakis C., Turpin V., Rossa P., Risjani Y., Sari L. A., Prasetiya F. S., Mouget J. L., 2019 Optimization of diatom *Haslea ostrearia* cultivation in different mediums and nutrients. IOP Conference Series: Earth and Environmental Science*,* 236:012044, 11 p.

Azmi M., Edward H. S., Andrio D., 2016 [Wastewater treatment of tofu liquid waste using *Typha latifolia* plant by using constructed wetland method]. Jurnal Online Mahasiswa (JOM) Fakultas Teknik 3(2):1-5. [In Indonesian].

Ballen-Segura M., Hernandez Rodriguez L., Parra Ospina D., Vega Bolanos A., Perez K., 2016 Using *Scenedesmus* sp. for the phycoremediation of tannery wastewater. Tecciencia 11(21):69-75.

Cheng W. H., Wong L. S., Chong K. C., 2019 Effect of parathion exposure towards green microalgae *Chlorella vulgaris* on the changes of physicochemical parameters in water. INTI Journal 2019:058, 6 p.

Chi Z., O'Fallon J. V., Chen S., 2011 Bicarbonate produced from carbon capture for algae culture. Trends in Biotechnology 29(11):537-541.

Dianursantia, Rizkytataa B. T., Gumelara M. T., Abdullah T. H., 2014 Industrial tofu wastewater as a cultivation medium of microalgae *Chlorella vulgaris*. Energy Procedia 47:56-61.

Dolatabadi S., Hosseini S. A., 2016 Wastewater treatment using *Spirulina platensis.* Journal of Chemical, Biological and Physical Sciences 6(4):1239-1246.

Ge S., Qui S., Tremblay D., Viner K., Champagne P., Jessop P. G., 2018 Centrate wastewater treatment with *Chlorella vulgaris:* simultaneous enhancement of nutrient removal, biomass and lipid production. Chemical Engineering Journal 342:310-320.

Han F., Huang J., Li Y., Wang W., Wan M., Shen G., Wang J., 2013 Enhanced lipid productivity of *Chlorella pyrenoidosa* through the culture strategy of semi-continuous cultivation with nitrogen limitation and pH control by CO_2. Bioresource Technology 136:418-424.

Husma A., 2017 [Biology of natural feeds]. CV Social Politic Genius (SIGn), Surabaya, Indonesia, 35 p. [In Indonesian].

Kang C. D., An J. Y., Park T. H., Sim S. J., 2006 Astaxanthin biosynthesis from simultaneous N and P uptake by the green alga *Haematococcus pluvialis* in primary-treated wastewater. Biochemical Engineering Journal 31(3):234-238.

Kong Q., Li L., Martinez B., Chen P., Ruan R., 2010 Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. Biotechnology and Applied Biochemistry 160:9-18.

Mardhia D., Abdullah V., 2018 [Study of water quality analyses in River Brangbiji Sumbawa Besar]. Jurnal Biologi Tropis 18(2):182-189. [In Indonesian].

Mohammed A. K., Ali S. A., Ali I. F., 2016 Using locally isolated *Chlorella vulgaris* in wastewater treatment. Engineering and Technology Journal 34(4):762-768.

Mufidah A., Agustono, Sudarno, Nindarwi D. D., 2017 [Culture technique of *Chlorella* sp. at laboratorium and intermediate scale in BPBAP Situbondo Jawa Timur]. Journal of Aquaculture and Fish Health 7(2):50-56. [In Indonesian].

Munir F., Hariyati R., Wiryani E., 2017 [Effect of tofu liquid waste on the growth of *Chlorella pyrenoidosa* H. Chick at laboratorium scale]. Jurnal Biologi 6(2):84-92. [In Indonesian].

Murray P. R., Rosenthal K. S., Pfaller M. A., 2016 Medical microbiology. Elsevier, 848 p.

Padang A., Lestaluhu A., Siding R., 2018 [The growth of phytoplankton *Dunaliella* sp. with different photoperiod at laboratorium scale] Jurnal Agribisnis Perikanan 11(1):1-7. [In Indonesian].

Paes C. R., Faria G. R., Tinoco N. A., Castro D. J., Barbarino E., Lourenço S. O., 2016 Growth, nutrient uptake and chemical composition of *Chlorella* sp. and *Nannochloropsis oculata* under nitrogen starvation. Latin American Journal of Aquatic Research 44(2):275-292.

Putri D., Ulhidayati A., Musthofa I. A., Wardani A. K., 2018 Single cell protein production of *Chlorella* sp. using food processing waste as a cultivation medium. IOP Conference Series: Earth and Environmental Science 131:012052, 7 p.

Safitri M. E., Diantari R., Suparmono, Muhaemin M., 2013 [Total fats of *Nannochloropsis* sp. at different photoperiods]. e-Jurnal Rekayasa dan Teknologi Budidaya Perairan 1(2):127-134. [In Indonesian].

Sepriani, Abidjulu J., Kolengan H. S. J., 2016 [The effect of liquid tofu wastewater to water quality in River Paal, District 4, Tikala Manado City]. Chemistry Progress 9(1):29-33. [In Indonesian].

Singh S. P., Singh P., 2015 Effect of temperature and light on the growth of algae species: a review. Renewable and Sustainable Energy Reviews 50:431-444.

Widayat W., Philia J., Wibisono J., 2018 Liquid waste processing of tofu industry for biomass production as raw material biodiesel production. IOP Conference Series: Earth and Environmental Science 248:012064, 5 p.

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