Liquid Waste Processing of Tofu Industry for Biomass Production as Raw Material Biodiesel Production

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Abstract. Indonesia as a producer of tofu generated more liquid waste. Nutrients that contained in the tofu wastewater are very useful for the production of microalgae. *Chlorella* sp. is a microalgalae that potential for food supplement, pharmaceuticals, animal feed, aqua culture and cosmetics. *Chlorella* sp. commonly grow in sea water. Glycerol in the cultivation medium as supplemental organic carbon sources for Microalgae. Cultivation carried out for 9 days at different percentages volume of tofu liquid waste and addition of glycerol. The result showed that increased concentration of tofu waste in microalgae cultivation media is proportional to the increase in lipid levels of *Chlorella* sp. Variable of 70%V has the highest concentration of lipid. The addition of glycerol in the cultivation medium enhancing lipid production. Variable of 5 g/l glycerol has the highest concentration of lipid. The higher ratio of carbon and nitrogen produces higher lipid content.

1. Introduction
Indonesia is one of the tofu producers that quite productive either home or industrial scale with a number of industry reaching 84,000 business units. For every 80 kg tofu produces 2,610 kg tofu wastewater. It means every year, for about 240 million people in Indonesia, 57,942 x 1.010 kg industrial tofu waste produced annually [1].

Gases commonly found in tofu waste are nitrogen (N$_2$), Oxygen (O$_2$), hydrogen sulphide (H$_2$S), ammonia (NH$_3$), carbon dioxide (CO$_2$) and methane (CH$_4$). The gases are derived from the decomposition of organic materials contained in tofu wastewater [2]. This time, tofu liquid waste is treated by anaerobic and aerobic methods. However, both of those method have several weakness. Anaerobic process have a weakness that is not able to eliminate nitrogen and phosphor, and requires the addition of a base for setting the basicity [3]. Aerobic method is able to eliminate the organic level and nutrients, but produces CO$_2$ and active mud that is the result of tofu waste treatment [4]. To solve the problem, there are methods to solve tofu wastewater as medium cultivation microalgae.

Nutrients contained in tofu waste are very useful for cultivation of microalgae. The protein in tofu wastewater occupies about 60% of all solid fractions. Through amino acids fermentation in anaerobic treatment process, protein can be degraded into various organic compounds [5]. Microalgae is one of various of photosynthetic organisms that found in the waters or places with a certain humidity [6]. Essential content of microalgae is used for some chemical products as food supplement, cosmetics, and pharmaceuticals [7]. Some microalgae is able to CO$_2$ fixation, sewage treatment industry, and produce lipids [6,8,9]. One of the microalgae that’s widely researched and developed this time is the
Chlorella sp. It contains proteins, carotenoids, lipids, immune stimulator compounds, polysaccharides, vitamins, antioxidants and minerals [10]. Lipids in Chlorella sp. can be extracted and processed to be fuel [11]. If Chlorella sp. is utilized as alternative fuel, the energy crisis problem in Indonesia might be answered.

In previous studies on Chlorella sp., various methods have been adopted to increase growth rate and algae concentration using seawater (nutrient composition, pH, light effect, temperature), using tofu wastewater as medium cultivation. Microalgae production with the addition of carbon sources is a viable means [12]. Glycerol in cultivation microalgae on tofu waste water is used for supplemental organic carbon sources. Therefore it is a need to conduct a research to know the effect on enhancing lipid production on cultivation microalgae types of Chlorella sp. in medium tofu wastewater.

Microalgae cultivation can be carried out with freshwater, seawater, water from estuaries, wastewater from agricultural, industrial operations and domestic wastewater [13]. All the microalgae media have several components in common: sources of nitrogen (in the form of nitrate, nitrite and ammonia), phosphorus, vitamins and trace metals. Some research suggests that tofu wastewater contains some nutrients which are very useful for the production of microalgae. Tofu wastewater contains some organic materials which mostly consist of proteins and lipids about 40-60% (226.06 mg/L to 434.78 mg/L) and other compound which are carbohydrate (25%-50%), and fat (10%) [14,15]. The wastewater also contains nitrate and phosphate [16]. Nutrients contained in the tofu wastewater are very useful for the production of Chlorella sp. Addition of glycerol on cultivation media will be carbon sources for microalgae [17].

2. Method

The material used in this research is tofu wastewater was obtained from tofu home industry in Mrican Village, inoculums of microalgae Chlorella sp. are obtained from BPAP Jepara that cultivated using water and fertilizer NPK, NaHCO$_3$ technical grade, B1, and B12 vitamin. N-hexane is required for lipid extraction. We need flask, aerator, neon lights, spectrophotometer Optima SP 300, salinity meter, glassware, Soxhlet to the research.

Research starts with the preparation of materials of tofu wastewater. Walter is filtered to reduce the total solid, then without sterilization, mix with water with ratio 1:9. Add nutrition 30 ppm to the mixture. Glycerol is added according to the variable (1 g/l, 3 g/l, 5 g/l, 7 g/l, 10 g/l). Chlorella sp. 10%V is added to the flask containing media with lighting and aeration for 9 days. Measure optical density is carried out every day by the spectrophotometry method using a spectrophotometer Optima SP 300 at a wavelength 680nm.

A mass of 2 gram of dried microalgae is used for lipid extraction with Soxhlet method.

| Factors               | Dimension (mm) |
|-----------------------|----------------|
| Overhang (OH)         | 3              |
| Pitch 1($p_1$)        | 3.5            |
| Pitch 2 ($p_2$)       | 4.5            |
| Pitch 3 ($p_3$)       | 3.5            |
| Thickness ($T_g$)     | 1.5            |
| Radius (R)            | 2.5            |
| Radius (R1)           | 2.3            |
| Height (h)            | 0.3            |
| Height (h1)           | 0.33           |
3. Results and Discussion

3.1 Effect of Tofu Wastewater Concentration in Medium to Lipid Production

Figure 1. Biomass production of *Chlorella* sp.

Based on Figure 1 shows that production of lipid has increased along with the increased of addition of tofu wastewater in media cultivation. Variable of 70% volume has the highest concentration of lipid. Microalgae uses organic substances in photobiosynthesis process. Tofu waste contains organic carbon that used for cultivation media. Increasing of tofu wastewater concentration is proportional to carbon content in cultivation media. The higher ratio of carbon and nitrogen produce higher lipid of *Chlorella* sp. [18].

3.2 Effect of Glycerol Concentration in Medium to Lipid Production

Figure 2. Lipid production of *Chlorella* sp.

Lipid contained in microalgae made up of glycerol and fatty acids. In previous experiment carried out on 2017, cultivation with 10% volume tofu wastewater as the medium has the best growth rate [19]. Figure 2 shows that the lipid level of *Chlorella* sp. with the addition of 1 g/l, 3 g/l, and 5 g/l glycerol has increased, but addition of 7 g/l and 10 g/l glycerol cause a decreasing level of *Chlorella* sp. Biosynthesis of TAG is a part of primary cell metabolism. TAG (Tryacylglycerol) is an ester of...
glycerol and three fatty acids. Tryglyceride is the main component of oil and fats. As a photosynthetic microorganisms, microalgae does the CO$_2$ fixation during the day and turn it into variety products, such as starch and TAG. The accumulation of starch and carbon decreases during the increases of nitrogen concentration. Carbon sources from glycerol convert into starch and TAG, so the addition of glycerol enhances lipid production of microalgae. Carbon partitioning can be affected by blocking the pathway of starch synthesis, leading to the diversion of carbon into TAGs and, hence, increased TAG yields [16]. Higher ratio of carbon and nitrogen in cultivation media produces higher lipid of Chlorella sp [8]. Accumulation of carbon metabolites into lipid on microalgae occurs when nitrogen content in cultivation media is small [20].

4. Conclusions
Addition of tofu waste and glycerol into the cultivation media effect on lipids production of Chlorella sp. Increasing concentration of tofu waste in microalgae cultivation media is proportional to the increase in lipid levels of Chlorella sp. Variable of 70%V has the highest concentration of lipid. Increasing of glycerol concentration cause higher production of lipids. Glycerol provides carbon sources as nutrient for microalgae. Carbon sources from glycerol convert into starch and TAG, so the addition of glycerol enhances lipid production of microalgae. Variable 5 g/l glycerol has the highest production of lipid.

5. References
[1] Herlambang, Arie 2008 Teknologi Pengolahan Limbah Cair Industri Tahu-Tempe access from http://www.kelair.bppt.go.id/Publikasi/BukuLimbahCairIndustri/02tempe.pdf (10 October 2018)
[2] Kaswinnarni, Fibria 2007 Kajian Teknis Pengolahan Limbah Padat dan Cair Industri Tahu
[3] Metcalf, E. dan Eddy, H. 2003 Wastewater Engineering: Treatment and Reuse (New York-Mc Graw Hill)
[4] Hongyang, S., Yalei, Z., Chunmin., Z., Xuefei, Z., dan Jinpeng, L 2011 Cultivation of Chlorella pyrenoidosa in Soybean Processing Wastewater Bioresource Technology 102 9884-9890.
[5] Ramsay I.R. and Pullammanappallil P.C., 2001 Biodegradation, Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry 12
[6] Mata, T. M., A. A. Martins, et. al. 2010 Microalgae for Biodiesel Production and Other Application : A Review Renewable and Sustainable Energy Reviews
[7] Borowitzka, M. A. 1999 Commercial Production of Microalgae : Ponds, Tanks, Tubes and Fermenters Biotechnology 313-321
[8] Wang B., Li Y, Wu N, Lan C.Q. 2008 CO$_2$ Bio-mitigation Using Microalgae Journal Applied Microbiology Biotechnology 79
[9] Christy, Y. 2005 Biodiesel from Microalgae Journal Biology Advances Elsevier 25
[10] Rekha Sharma, Gajendra Pal Singh, Vijendra K. Sharma, Plant Pathol Microb 2012 Effect of Culture Condition on Growth and Biochemical Profile of Chlorella vulgaris 3 5
[11] Blinova, Lenka, Alica Bartosova, Kristina Gerulova. 2015 Cultivation of Microalgae (Chlorella vulgaris) for Biodiesel Production Research Papers Faculty of Material Sciences and Technology in Trnava 23
[12] Ma, Xiaochen, Hongli Zheng, Min Addy, Erik Anderson, Yuhuan Liu, Paul Chen, Roger Ruan 2016 Cultivation of Chlorella vulgaris in Wastewater with Waste glycerol: Strategies for Improving Nutrients Removal and Enhancing Lipid Production Accepted Manuscript
[13] Luisa F. Rios and others, 2015 Influence of Culture Medium on Desmodesmus sp. Growth and Lipid Accumulation for Biodiesel Production Chemical Engineering Transactions 43 601-606
[14] Nurhasan and Pramudiyanto 1991 Penanganan Air Limbah Tahu yayasan Bina Karya Jakarta Selatan, acces from http:www.menlh.go.id (24 september 2008)
[15] Singh J, Gu S. 2010 Commercialization Potential of Microalgae for Biofuels Production Renewable and Sustainable Energy Reviews 14 2596-2610
[16] Liu, Bensheng dan Christoph Benning 2012 Lipid Metabolism in Micoralgae Distinguishes Itself
Current Opinion in Biotechnology 24 300-309

[17] Kong, Wei-Bao, Hong Yang, Yun-Tao Cao, Hao Song, Shao Feng Hua, Chun-Gu Xia 2012 Effect of Glycerol and Glucose on The Enchancement of Biomass, Lipid and Soluble Carbohydrate Production by Chlorella vulgaris in Mixotrophic Culture Food Technology and Biotechnology 51 1-11

[18] Wang B., Li Y, Wu N, Lan C.Q. 2008 CO₂ Bio-mitigation Using Microalgae Journal Applied Microbiology Biotechnology 79

[19] Widayat, W., John Philia, Jessica Wibisono 2017 Cultivation of Microalgae Chlorella sp on Fresh Water and Waste Water of Tofu Industry E3S Web of Conferences 31 00409

[20] Yeesang dan Cheirsilp B 2011 Effect of Nitrogen, Salt, and Iron Content in The Growth Medium and Light Intensity on Lipid Production by Microalgae Isolated from Freshwater Sources in Thailand Journal Bioresource Technology 102 3034-3040.

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