Industrial Tofu Wastewater as a Cultivation Medium of Microalgae *Chlorella vulgaris*

Dianursanti\textsuperscript{a,}*\textsuperscript{*}, Baharudin Taufiq Rizkytata\textsuperscript{a}, Mohamad Teguh Gumelar\textsuperscript{a}, Taufik Hidayat Abdullah\textsuperscript{a}

\textsuperscript{a}Bioprocess Technology, Chemical Engineering Department, Faculty of Engineering, University of Indonesia, Gedung GK Lantai 1, Faculty of Engineering, University of Indonesia, Depok 16424

**Abstract**

*Chlorella vulgaris* have high lipid productivity as biodiesel source. Cultivation of *C. vulgaris* in this study using tofu wastewater in a 18-liters photobioreactor, 5 000 lux illumination, the pressure 1 atm and a temperature is 28°C. The results indicated that the optimum of Chlorella growth found in cultivation by 20-30% waste medium. Waste concentration that higher than 35%, elucidated a decreased Chlorella growth. Amount of lipids obtained from cultivation using the waste medium was approximately 23%. Reduction of BOD, COD, phosphates, and ammonia ions are lower than the threshold value of waste after the cultivation is done.

**Keywords**: *Chlorella vulgaris*; cultivation; medium; tofu wastewater; lipid

**Nomenclature**

| Symbol | Definition |
|--------|------------|
| X      | cell dry weight (g/L) |
| N      | number of cell density (cell/L) |
| \(\mu\) | specific growth rate |
| OD     | optical density of cell |

* Corresponding author. Tel.: +62817-9142183

\textit{E-mail address}: danti@che.ui.ac.id
1. Introduction

Chlorella vulgaris has been exploited recently to produce lipid as biodiesel material, since it has higher lipid content compared to other algae as shown in table 1. It also has been reported that C. vulgaris able to degrade pollutants and grow in community domestic wastewater [1], and agro industrial wastewater.

Table 1. Lipid content comparison in percent [3].

| No | Algae                | Lipid Content |
|----|----------------------|---------------|
| 1  | Chlorella vulgaris    | 14%-22%       |
| 2  | Scenedesmus obliquus  | 12%-14%       |
| 3  | Dunaliella bioculata  | 8%            |
| 4  | Spirulina plantesis   | 4%-9%         |

C. vulgaris ability to grow in poor medium [2] and waste create a possibility for its usage in waste treatment. Information was gathered from previous research led to a conclusion that C. vulgaris were able to degrade pollutants in its medium such as ammonia, nitrate, phosphate and heavy metals. Furthermore, C. vulgaris can tolerate CO₂ concentration up to 40% and has high lipid content as biodiesel source hence it can reduce greenhouse gases effects [3]. Regarding those information, this paper will describe the influence of industrial tofu wastewater as a cultivation medium to the growth rate and lipid content of microalgae C. vulgaris as biodiesel source.

Industrial tofu wastewater available in large quantity in Indonesia. Tofu consumption level in Indonesia reached 7.4 kg/person/year. Data obtained from BPPT (Agency for the Assessment and Application of Technology) showed that for every 80 kg tofu produced, 2 610 kg waste produced. It means every year, for about 240 million people in Indonesia, 5.7942 x 10¹⁰ kg industrial tofu waste produced annually. The wastewater produced contains some organic materials which mostly consist of proteins and lipids about 40-60% (226.06 mg/L to 434.78 mg/L) [4] and other compound which are carbohydrate (25%-50%), and fat (10%) [5]. The wastewater also contains nitrate and phosphate [4]. This led to a possibility for C. vulgaris to grow in industrial tofu wastewater medium.

To utilize industrial tofu wastewater as cultivation medium of Chlorella vulgaris, suitable composition of the medium needs to be determined scientifically. The influence of industrial tofu wastewater as a cultivation medium to the growth rate and lipid content of microalgae C. vulgaris described in this research. Cultivation effect on pollutants in the wastewater also will be given.

2. Material and method

2.1 Reactor

Reactor used is plate photobioreactor 18 Liters in volume. The reactor is closed to prevent contamination from the environment and equipped with additional devices, which is aeration of CO₂ and air 5 L/min, and artificial illumination about 5 000 lux using Phillip Hallogen lamp 23W/12V/50Hz. Sterilization also done using suitable method.

2.2 C. vulgaris pure culture

C. vulgaris used kindly provided by Freshwater Fish Research Center in Depok. Pure culture control were cultivated by using Walse medium, which promote highest lipid content compared to other chemical medium [6].

2.3 Tofu wastewater medium preparation

Medium prepared in 5 variation (shown in table 2) by diluting the wastewater using pump water which had been sterilized using autoclave for 15 minutes.
Table 2. Tofu wastewater medium variation.

| Wastewater volum (%n) | Medium volume (L) | Wastewater : total volume |
|-----------------------|-------------------|--------------------------|
|                       | Pump Water        | Wastewater               |
| 10.0                  | 10.8              | 1.2                      | 1 : 14.0                |
| 20.0                  | 9.6               | 2.4                      | 1 : 6.5                 |
| 30.0                  | 8.4               | 3.6                      | 1 : 4.0                 |
| 37.5                  | 7.5               | 4.5                      | 1 : 3.0                 |
| 50.0                  | 6.0               | 6.0                      | 1 : 2.0                 |

C. vulgaris were cultivated in all medium variation for 204 hours.

2.4 Growth measurement

Biomass production rate was observed by using optical density data of the colony in the reactor for every 6 hours in 204 hours using UV-VIS RS Spectrometer, LaboMed. Inc. The OD data obtained converted to dry biomass using following equation [7]:

\[
X = 0.551(OD) + 0.138 \tag{1}
\]

\[
\mu = \frac{1}{x} \frac{dX}{dt} \quad \text{or} \quad \frac{dN}{dt} \tag{2}
\]

where \(X\) represent dry biomass, \(OD_{600}\) is optical density obtained at \(\lambda = 600\)nm, \(\mu\) represent specific growth rate, \(N\) means cell number, and \(t\) is time.

2.5 Lipid content measurement

Lipid extracted from algae using Bligh Dryer Method. The microalgae separated from medium by gravity. Every 1 ml cake mixed with 2 ml methanol and 1 ml chloroform in Erlenmeyer flask then sonicated. After mixed well, 1 ml of chloroform and 1 ml of aquadest added. After that, the mixture sonicated again. Well-mixed mixture centrifuged for 10 minutes, 8 500 rpm resulting 2 layer mixture. Lipid separated from the mixture then dried, and dry lipid content measured.

3. Result and discussion

Optical density data obtained from the culture cultivated in industrial tofu wastewater processed and plotted against time. The higher \(OD_{600}\) implicates more cell existed in the sample analyzed. Analysis result showed in figure 1.

![Fig.1 Growth curve of Chlorella vulgaris in medium (A) Walne, (B) 10% tofu wastewater, (C) 20% tofu wastewater, (D) 30% tofu wastewater, (E) 37.5% tofu wastewater, (F) 50% tofu wastewater.](image-url)
Every culture OD started at around 0.2 based on OD data obtained, medium containing 20% and 30% industrial tofu wastewater depict growth curve similar to which cultivated in Walne medium. Biomass obtained from medium with 20% and 30% industrial tofu wastewater is 0.758 g/L and 0.827 g/L respectively while Walne medium gives biomass 0.747 g/L. As shown in figure 1, microalgae growth in Walne medium (A) is stable, while it is fluctuate in tofu wastewater (B). This is happened probably because Walne medium contains micronutrients, which are Fe, Mn, Mg and Cl. For medium with 20% tofu wastewater (C), highest biomass obtained is 0.674 g/L since the medium has fewer nutrients compared to medium with 30% tofu wastewater (D). Microalgae growth in medium 20% tofu wastewater shows decrease in growth after 144 hours of cultivation. In medium with 10% wastewater, biomass obtained is 0.452 g/L and the microalgae can survive only for 48 hours.

As shown in figure 1, time needed by *Chlorella vulgaris* to pass adaptation phase depend on wastewater concentration in medium. This is related to the number of protein and carbohydrate particles in the medium. As the number of particles gets higher, time needed to pass adaptation phase become longer. The number of particles can be indicated from turbidity, BOD and COD value. In higher turbidity, photosynthesis becomes more inhibited due to smaller light intensity received, resulted *C. vulgaris* grow slowly. Previous study shows optimum light intensity needed by *C. vulgaris* to grow is 5 000 lux [7].

Initial pH of wastewater used in this experiment is pH 5. This acidic properties leads to the decrease of the initial pH of medium used as wastewater concentration gets higher. Data from experiments elucidated increase of pH from initial condition over time. Maximum *C. vulgaris* growth observed at pH of 6 – 7. When pH of medium exceeds 8, *C. vulgaris* growth decreases significantly. Acid condition or basic environment affected cell physiology and can leads to inhibition of cell growth.

Our experiment shows *C. vulgaris* cannot survive in medium containing 37.5% and 50% wastewater. This is due to high turbidity, BOD and COD value. High turbidity makes light hard to pass the medium. These concentration caused to pH of the medium is decreasing over time. higher contamination suspect as the main reason of *C. Vulgaris* death and also contributes to the decrease in pH.

Highest growth rate observed at wastewater medium concentration of 30% compared to other concentration variation. Even the growth rate in 10% and 20% wastewater concentration medium is still higher than *C. vulgaris* cultivated in Walne medium. Highest growth rate in 30% wastewater concentration medium reached after 90 hours. At this medium, adaptation phase take longer time.

Nitrogen and phosphor in the medium is in the form of ammonium ion and phosphate ion. These ions can be used directly for microalgae metabolism. Otherwise, in Walne medium nitrate must be converted to ammonium ion before it can be used by microalgae. Ammonium ion is needed in photosynthetic process and affect essential content of microalgae. Phosphate ion in the medium needed for energy metabolism, cell membrane stabilization, carbohydrate biosynthesis, amino acid biosynthesis and cell replication. If photosynthesis product is decreasing, carbohydrate left in microalgae after respiration process will not be sufficient for cell replication.

Essential content tested in this research is only lipid. The analysis of lipid content due to lipid can be synthesized to biodiesel, as renewable energy. Lipid contained in microalgae usually is in the form of glycerol ester and fatty acid with 14 – 22 carbon atoms [8]. As shown in figure 2, medium concentration of 20% and 30% yield highest lipid content, 22.965% and 23.249%, respectively. This result is relatively same with Walne medium. At medium concentration of 10%, biomass production is low and the lipid content is only 14.460%. This productivity relatively correlated to theoretical lipid content of *C. vulgaris* that is 14% – 22% [8].

Lipid content in microalgae is highly depend on nitrate in the medium. Even ammonium ion content in wastewater medium is relatively small, lipid content after cultivation is relatively same with Walne medium. As nitrogen compound in Walne medium converted to ammonium ion higher, lipid production will decrease because more NADH is needed. The average of ammonium ion needed by microalgae is about 5% – 10% of its dry weight or about 5 – 50 mM [6].
Analysis of industrial tofu wastewater is given in table 3. Table 3 shows the fact that tofu wastewater has phosphate content, ammonia content, with high COD and BOD value. Before cultivation, BOD and COD level exceeds Indonesian government standard. After cultivation, BOD and COD decrease to the level below Indonesian government standards threshold. Phosphate, ammonia, and total nitrogen content still meet Indonesian government standard.

BOD and COD value fall significantly after cultivation. During cultivation, protein and lipid particle is broken down into simpler compound and absorbed by *C. vulgaris* as nutrition. Phosphate and ammonia used by *C. vulgaris* in metabolism process. Significant change also happened in pH of the medium. Wastewater medium condition change from relatively acidic into basic after cultivation.

| Parameter   | Units | Before Cultivation | After Cultivation | Indonesian Government Standard |
|-------------|-------|--------------------|-------------------|-------------------------------|
| Phosphate   | mg/L  | 1.06               | 0.80              | 3.00                          |
| Free ammonia| mg/L  | 0.05               | 0.04              | 10.00                         |
| BOD₅        | mg/L  | 4.23               | 89.30             | 125.00                        |
| COD         | mg/L  | 9.74               | 176.00            | 300.00                        |
| N-total     | mg/L  | 8.74               | 0.56              | 25.00                         |
| pH          |       | 5.00               | 9.00              | 6.00 – 9.00                   |

3. Conclusion

Growth curve at medium concentration of 20% and 30% relatively similar with Walne control medium. This is due to ammonium ion and phosphate ion that can be used directly in metabolism. *C. vulgaris* cannot survive in medium concentration of 37.5% and 50% because of high level of BOD and COD. More acidic condition over cultivation time also contributes to the death of *C. vulgaris*. Relatively similar lipid productivity obtained at medium concentration of 20%, 30% and control medium. BOD, COD, phosphate, free ammonia, N-total and pH parameter of tofu wastewater after cultivation meet Indonesian government standard.
Acknowledgements

Our research was funded by Indonesian Ministry of Education as a part of PKM (Student Creativity Program) research program.

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