Growth of locally isolated microalga in POME to produce lipid as alternative energy sources

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Abstract. Purpose of this study was to find the best growth of locally isolated microalgae that produce lipids from Palm Oil Mill Effluent (POME) as an alternative energy source. Microalgae was cultivated in POME in glass vessel at room temperature using a lighting intensity of 13,000 lux and continuously aeration for 24 and 12 hours, respectively. Biomass of microalgae were analyzed daily to get their growth by spectrophotometry at 624 nm wavelength, whereas Modified Bligh and Dyer method determined lipid content. Results show that the best growth occurred at 10% inoculum with lighting cycle and aeration of 24 hours (on/off) and resulting highest biomass content of 0.99 g dry weight/L followed by the decrease of organic substances in POME. The percentage reduction of COD, BOD, TSS, and oil at POME reached above 92%, while phosphate concentration reached 89.2%. Cultivation of microalgae in POME for 12 days showed its ability to reduce organic substances and nutrients in POME and produced biomass with lipid content of 35%. These results reached to the conclusion that locally isolated microalgae has an ability to treat POME safely for environment and POME can be used as a growing medium of microalgae that produces lipids.

1. Introduction

Environmental pollution has been a big issue in recent years caused by the continuous disposal of wastewater in large volumes, resulting a decrease in water quality such as decreased dissolved oxygen in water, increased nutrients, and odors, even causing the surrounding water to be unusable. In some developing countries, palm oil industry continues to grow rapidly followed by the increased wastewater from Palm Oil Milling Effluent (POME), which can have a negative impact on the environment. Generally, the palm oil mill entrepreneurs consider that wastewater treatment is very expensive, requires complicated technology, and has no economic value. Various renewable technologies can be applied dealing with water pollution issues that continue to degrade due to disposal of industrial wastewater, especially from palm oil industries. The increasing POME wastewater from palm oil industry is also one of the causes of greenhouse gases; therefore, POME wastewater treatment is needed for the environment and more effective and sustainable.

The use of microalgae for wastewater treatment offers several advantages over traditional waste treatment, including cost-effectiveness in removing BOD, phosphors, nitrogen, and removal of pathogenic bacteria compared to active sludge systems. Through the process of wastewater treatment using microalgae will produce biomass in large numbers [1]. Naturally, microalgae works to reduce concentration of nitrogen and phosphors in wastewater [2]. In the uncontrolled state, residual
wastewater entering the waters can lead algal growth that known as algal bloom and eutrophication. A very common method for eliminating nitrogen is through denitrification process that reduces nitrates to gases nitrogen and released it into the atmosphere [3].

Liquid waste from palm oil industry is generally rich in nitrogen, phosphor, carbon, and sulfur nutrients which are nutrients for microalgae growth [4]. Processing ponds of microalgae have potential to reduce nitrogen and phosphorus nutrients through assimilation into microalgae biomass followed by harvesting. The performance of microalgae is better than that of bacteria in lowering COD concentration because microalgae can grow in water contains low carbon. This is due to its ability to utilize dissolved carbon from the air [5]. Wang et al. [6] demonstrated the feasibility of cultivation of Chlorella sp. to treat wastewater from settlement that Chlorella sp. can adapt well without experiencing lag phase. Algae growth is increasing rapidly in the middle side of processing pond due to the high content of nitrogen, phosphor, and COD. Algae biomass is also highly efficient in degrading waste into carbon (zero waste) and producing high density of liquid energy [7]. The use of microalgae for wastewater treatment offers several advantages, namely microalgae provide an effective way to consume liquid waste nutrients and provide enough oxygen for aerobic bacteria through photosynthesis.

Those studies have prompted researchers to examine locally isolated microalgae growth in POME wastewater. Based on the concept of sustainable and environmentally development, POME wastewater can be utilized as nutrients for the growth of microalgae in reducing organic substances and microalgae biomass utilized as alternative energy sources in an effort to overcome problems of impending energy deficit. In an effort to cost reduction of biodiesel production, it requires use of low-cost raw materials that produce alternative energy and at the same time reduce the potential environmental pollution due to waste disposal [8-10].

This paper describes the growth of locally isolated microalgae in reducing organic compounds in POME wastewater as an effort to obtain alternative energy source. It is expected that this research can contribute as a reference of POME treatment system in obtaining alternative energy sources, so the realization of sustainable and integrated industrial development can solve the problem of energy deficit in the future.

2. Materials and Methods

2.1 Materials

POME liquid waste samples obtained from the oil palm industry in Nagan Raya District, Aceh were precipitated and screened before being used in the treatment. The concentration of POME prepared for this research was 25% (LP25). Local microalga used was a stock culture of microbiology laboratory that has been isolated from the open pound of Banda Aceh City.

2.2 POME characteristics analysis

Characteristics of POME was evaluated by measuring several key parameters such as COD, BOD, TSS, oil content, and NH4, NO3 (total-Nitrogen), and PO4 using standard methods APHA [11]. At the end of culturing, the inoculum was re-analyzed to see the decrease of organic compounds in POME with the same parameters.

2.3 Cultivation of locally isolated microalgae

Ten percent of microalgae inoculum (v_inoculum/v_media) was cultivated in POME (LP25) in 1500 ml glass vessel at room temperature using a lighting intensity of 13,000 lux and continuously aeration for 24 and 12 h, respectively. Biomass of microalgae were analyzed daily to get their growth by determining the content of algae biomass (in dry weight), whereas characteristic POME wastewater and lipid content were analyzed at the end of cultivation.
2.4 Culture analysis
Microalgae growth curve was obtained by determining the weight of dry biomass. The growth of microalgae was analyzed by spectrophotometry at 624 nm wavelength, whereas lipid content was determined by modification method of Bligh and Dyer [12]. Microalgae cells were extracted with 2:1 (v/v) chloroform and methanol solution, the solution would be separated into a chloroform and methanol layer by addition of methanol and water to give the final solvent ratio of chloroform:methanol:water (1:1:0.9). Chloroform layer was washed with 20 mL of 5% NaCl, evaporated to dryness, and lipid total was determined gravimetrically.

3. Results and Discussion

3.1 Influence of inoculum concentration on biomass growth of microalgae in POME wastewater
Locally isolated microalgae was applied in POME wastewater to reduce organic substances contained in POME in order to dispose of the wastewater safely into the environment. Figure 1 shows the characteristics of locally isolated microalgae growing in POME wastewater during 12 days cultivation. The growing microalgae in POME wastewater have the ability to adapt to highly concentrated POME and high organic content.

![Figure 1. Effect of inoculum concentration on biomass growth of microalgae in POME](image)

Figure 1 shows that the best microalgae growth was obtained from a 10% inoculum concentration in the 24-hour lighting and aeration cycle (on/off) which produced highest biomass of 0.34 g-dry weight/L on day 10, whereas the highest biomass content at 20% inoculum concentration reached only 0.26 g-dry weight/L. That was due to the inoculum of microalgae not acclimatized, so the growth of microalgae was very slow in adjusting to concentrated POME concentration with high organic matter content, takes long time for next growth phase, and yields less biomass. Growth of microalgae was strongly influenced by growing media used, acclimatization of inoculum needs to be done so that POME can be a good growth medium for local isolate microalgae which has potential as alternative media of mass algae growth. According to Bertoldi, et al. [13], alternative media culture has been evaluated for microalgae cultivation such as industrial and agricultural wastewater containing nutrient-rich residues as nutrients for aquaculture biomass growth. Wastewater concentration is very influential on the growth of microalgae, higher concentration of wastewater occurs growth of microalgae in the lag phase slower because it needs to take adaptation time to grow.
3.2 Effect of lighting cycle and aeration on microalgae growth in POME wastewater

Growth of microalgae was strongly influenced by carbon dioxide, lighting cycles, nutrients, and cultivation time. Figure 2 shows that microalgae biomass content was higher within 24 hours lighting and the aeration than 12 hours lighting and aeration with the same lighting intensity of 13000 lux. Furthermore, Figure 2 shows that the best microalgae growth was obtained from a 10% inoculum concentration in the 24-hour lighting and aeration cycle (on/off) which produced highest biomass of 0.99 g dry weight/L on 5 day. The next day, biomass growth gradually decreased to 12 day. The reduced of lighting cycles and nutrient content in wastewater caused the process of photosynthesis running slowly and producing less microalgae biomass.

![Figure 2. Effect of lighting cycle and aeration on microalgae growth in POME](image)

3.3 Reduction of organic substance and nutrient on POME by microalgae

POME wastewater with high content of organic substances began to be degraded well by microalgae after 2 days of cultivation. In the adaptation phase, microalgae can survive to lag phase so nutrients as their food sources in the wastewater gradually decreased. Reduction of nutrients in wastewater were in line with the decreasing of organic substances in POME.

Yunaridi [5] reported that the performance of microalgae is better than that of bacteria in lowering COD concentration because microalgae can grow in water contains low carbon. This is due to its ability to utilize dissolved carbon from the air. Table 1 shows the ability of microalgae to remove organic substances and nutrients in POME wastewater for 12 days. The analysis result shows that microalgae have the ability to decrease organic and nutrient substances in POME reach 92%. It was indicated from the decreasing of COD, BOD, TSS, and oil concentration, whereas phosphate concentration reach 89.2% and ammonium concentration was only 32.4%. Cultivation of microalgae in POME wastewater at LP25 concentration for 12 days showed its ability in reducing organic, nutrient, and yielding biomass with a total lipid content of 35%. Algae biomass was very efficient in transforming waste into carbon (zero waste) and producing high-density liquid energy [7]. Culturing process and destruction of biomass cells was very influential on the release of lipids contained in microalgae biomass. Lipid extraction with Modified Bligh Dyer [12] can extract more lipids contained in microalgae biomass because the solvent ratio can help the extraction of all lipids contained in the biomass.
Table 1. Characteristic of POME after treatment using locally isolated microalgae

| No | Parameter                  | Before treatment (mg/L) | After lighting and aeration treatment |
|----|----------------------------|-------------------------|---------------------------------------|
|    |                            |                         | 12 hours  | 24 hours  |
|    |                            |                         | mg/L     | %         | mg/L     | %         |
| 1  | BOD                        | 5713                    | 1660     | 70.9      | 335.5    | 94.1      |
| 2  | COD                        | 38883                   | 6532     | 83.2      | 2820     | 92.7      |
| 3  | Suspended Solids           | 16800                   | 7850     | 53.3      | 185.0    | 98.9      |
| 4  | Phosphate (PO₄)            | 536.29                  | 75.5     | 86.0      | 58.0     | 89.2      |
| 5  | Ammonium (NH₄)             | 77.4                    | 52.3     | 32.4      | 121.7    | (57.2)    |
| 6  | Oil contents               | 256.0                   | 33.0     | 87.1      | 1.6      | 99.4      |
| 7  | Nitrate (NO₃-N)            | 49.0                    | 80.0     | (63.3)    | 56.0     | (14.3)    |

Condition: Inoculum 10%, light intensity 13,000 lux, POME concentration 25% (LP₂₅)

4. Conclusions
The best locally isolated microalgae growth was obtained at 10% inoculum concentration with lighting cycle and aeration of 24 hours (on/off) and resulting highest biomass content of 0.99 g dry weight/L followed by the decrease of organic substance in POME wastewater. The percentage reduction of COD, BOD, TSS, and oil at POME reached above 92%, while phosphate concentration reached 89.2%. Cultivation of microalgae in POME wastewater at LP₂₅ concentration for 12 days showed its ability to reduce organic substances and nutrients and produced biomass with a total lipid content of 35%.

Authors Contribution: Erman Munir, Delvian, and Hesti Wahyuningsih contributed to the discussion of the results.

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