Production of Lipid Biomass in Locally Isolated Microalga Cultivated in Palm Oil Mill Effluent (POME)

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Abstract

Purpose – This study aims to obtain the best lipid content in locally isolated microalga that grows in palm oil mill effluent (POME).

Design/Methodology/Approach – Microalgae were cultured in POME with 25% dilution (LP25), 50% (LP50), and no dilution (LP) in 1,500 ml glass vessel at room temperature using a lighting intensity of 13,000 lux and continuous aeration for 24 hours and 12 hours, respectively. The biomass (in dry weight) of microalgae was analyzed daily by means of spectrophotometry using 624 nm wavelength to determine their growth.

Findings – The results showed that the acclimatized growth of microalgae in POME media adapted faster to the POME concentration. Acclimatized biomass content tends to increase to 1.014 g/L, while the content of non-acclimatized biomass reached only 0.752 g/L. Lipid content resulting from the direct extraction process using the modified Bligh and Dyer method provided the best yield of 47% in the microalgae grown in the LP50 POME medium.

Research Limitations/Implications – Lipid was produced from locally isolated microalgae cultured in POME medium with 25% dilution (LP25), 50% (LP50), and no dilution (LP).

Practical Implications – The lipid produced had the potential for biodiesel energy.

Originality/Value – In this study, microalga was used not only to treat POME liquid wastes but also to produce lipids as biodiesel energy potentials.

Keywords Lipid content, POME, Biomass

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1. Introduction

Palm oil industry in Indonesia continues to grow rapidly resulting in the increased production of palm oil followed by the increase of Palm Oil Mill Effluent (POME) that causes negative impact on the environment. Prevention and mitigation of the negative impacts of POME’s liquid waste continue to be in line with the efforts to improve its positive impact.
POME is a non-toxic waste product obtained from palm oil industry, which is highly polluted due to the presence of organic matter with Chemical Oxygen Demand of 50,000 mg/L and total nitrogen content of 750 mg/L. Wong et al. (2009) reported that this liquid waste is acidic in nature and has brownish color with 95–96% of water content, 4–5% total solid including 2–4% solid suspension, as well as 0.6–0.7% oil and fat.

Due to the limited carrying capacity of the natural environment in neutralizing POME, it is necessary to overcome the problem of pollution by using integrated preventive measures. POME can be utilized as a medium of microalgae growth which has economic value and potential as an alternative energy to overcome problems of the future energy deficit. The decline of fossil fuel reserves in the world due to high economic growth has resulted in the decrease in the availability of non-renewable energy. High level of environmental pollution due to the use of fossil fuels and edible vegetable oils for biodiesel has led to competition with food needs, so it is not feasible to be developed on a large scale (Knothe, 1997). Unlike other seed crops, microalgae have the potential to replace conventional fuel oil use; they can grow fast and have very high oil content. Generally, microalgae can reproduce in 24 hours (Chisty, 2007; Abou-Shanab et al., 2009; Teresa et al., 2010). Studies for finding alternative energy sources still continue and the use of microalgae as a source of energy has attracted the attention of researchers.

Microalgae is one of the best raw materials that can produce lipids as a source of biodiesel energy, because microalgae have several advantages, namely: high photosynthetic efficiency, high biomass production, and faster growth than other food crops. Microalgae not only have the capacity to produce valuable products, but also have the ability to multiply using only sunlight and carbon dioxide. They also act as a fixture of greenhouse gases or CO₂ in the air, thereby lowering CO₂ levels that are thought to be causing global warming. It has a highly efficient biomass that takes carbon (zero waste) in the form of waste and converts it into a form of high-density liquid energy (Grima et al., 2003; Widjaja et al., 2008).

Reducing the cost of biodiesel production requires low-cost raw materials for producing environmentally friendly energy and at the same time reduce the potential for environmental pollution due to waste disposal (Ghaly et al., 2010). Liquid waste from agroindustry is generally rich in N (Nitrogen), P (Phosphor), C (Carbon), and S (Sulfur) nutrients which are nutrients for microalgae cell growth. Processing ponds of microalgae have a potential to remove phosphorus and nitrogen nutrients through assimilation into algal biomass followed by harvesting of biomass.

Zhu et al. (2001) have extracted mortiella alpine oil by the Bligh Dyer method and it is known that dry biomass provides higher oil yields than wet biomass. Algae oil extraction is a more efficient way for obtaining energy than fermenting algae biomass into methane or methanol. The content of oil and fatty acid in microalgae vary according to the conditions of cultivation. Other researchers have also extracted Chlorella protothecoides microalgae oil using n-hexane solvents and further processed them into biodiesel using an acid catalyst. Further, Lee et al. (2010) have also studied the comparative destruction of microalgae cells prior to the extraction method for obtaining lipids and reported that the method of cell destruction using microwave was a very simple, easy, and efficient method of cell destruction and produces the highest lipid.

Several researches have been undertaken to develop techniques, procedures, and microalgae production processes in large quantities. Open ponds system and photo-bioreactor system is a technique of microalgae cultivation that is often used. Photo-bioreactor system was developed to overcome the problems of contamination and evaporation that often occur in Open Ponds system. This type of flat plate is often used instead of the tubular
type because it can flatten the intensity of irradiation and produce high-density cells (Lee et al., 1998). In this study, we have tried to obtain the best lipid content from locally isolated microalgae grown in POME media.

2. Materials and method

2.1. Materials

POME obtained from PT. Fajar Bayzury & Brothers in Nagan Raya District, Aceh, and the stock sample of local isolated microalgae was isolated from open ponds in Banda, Aceh City. Before treatment, POME was precipitated and filtered, followed by characterization with COD, BOD, TSS, oil, \( \text{NH}_4 \), \( \text{NO}_3 \) (total-Nitrogen), and \( \text{PO}_4 \) parameters using the APHA method (1999).

2.2. Research procedure

2.2.1. Cultivation of locally isolated microalgae. Twenty percent of microalgae were cultured in POME with 25\% dilution (LP\(_{25}\)), 50\% (LP\(_{50}\)), and no dilution (LP) in 1,500 ml glass vessel at room temperature using a lighting intensity of 13,000 lux and continuous aeration for 24 hours and 12 hours, respectively. Biomass (in dry weight) of microalgae were analyzed daily by means of spectrophotometry using 624 nm wavelength to determine their growth, whereas characteristic POME and lipid content were analyzed at the end of cultivation.

2.2.2. Lipids analysis. Lipid content was determined by the modified Bligh and Dyer (1959) method, the generated microalgae biomass was destroyed by a mortar and carried into a separator funnel, then extracted using a solution of chloroform 2:1 methanol (v/v) and separated into layers of liquid chloroform and methanol by the addition of methanol and water to produce the final solvent ratio of chloroform:methanol:water of 1:1:0.9. The chloroform layer was washed with 20 ml solution of 5\% NaCl, and evaporated until dry, and the total lipid was determined using the gravimetric method. After obtaining the lipid weight, the yield of lipids was calculated using the following equation.

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\text{Yield} = \frac{\text{oil weight}}{\text{dry biomass weight}} \times 100\%
\]

3. Results and discussion

3.1. Influence of POME concentration to the growth of microalgae

The non-acclimatized microalgae in different concentrations of POME were cultured in a fixed state at room temperature and continuous intensity of 24 hours, as shown in Figure 1. The microalgae growth was found to be very slow in the LP\(_{25}\) POME medium, because it was difficult to adapt to this concentration of POME and need long time for growth. On the other hand, the growth phase of microalgae in LP\(_{50}\) POME medium was faster, which within 8 days and produced biomass content of 0.75 g/L. POME media with certain nutrient concentrations can be an alternative potential growing medium for massive microalgae growth in the future (Habib et al., 2003). Industrial and agricultural liquid waste has been evaluated as an alternative to nutrient-rich culture media for the cultivation of microalgae (Bertoldi et al., 2006).

3.2. Influence of acclimatization to the microalgae growth

The acclimatized and un-acclimatized microalgae growth in the LP\(_{50}\) POME medium shows a very different curve, as shown in Figure 2. The non-acclimatized microalgae growth curve in
POME media still has to adjust to the organic matter concentration contained in the liquid waste, whereas the acclimatized microalgae can more quickly adjust to the POME concentration.

3.3. Influence of growth microalga to the yield of lipid
The ability of microalgae in producing total lipids is shown in Figure 3. Microalgae lipid content was produced from direct extraction process using the modified Bligh and Dyer (1959) method. This method produces more efficient energy from microalgae than the method of fermentation into methane (Zhu et al., 2001). The formation of lipid content was determined by microalgae growth conditions, such as light, nutrient, aeration, etc. Figure 3
shows that a better total lipid content (yield) was obtained from the microalgae grown in POME with LP$_{50}$ concentration. The process of culturing and destruction of biomass cells was very influential on the release of lipids contained in microalgae biomass.

4. Conclusion
This research concludes that the acclimatized microalgae growth in POME media was faster to adapt to POME concentration. Acclimatized biomass content tends to increase to 1.014 g/L, while the content of non-acclimatized biomass reached only 0.752 g/L. Lipid content provided the best yield of 47% in the microalgae grown in the LP$_{50}$ POME medium.

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