Aerobic-pond Palm Oil Mill Effluent (POME) utilization as growth medium of *Scenedesmus obliquus* for lipid production

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Abstract. The purpose of this research is to know the growth rate and biomass production of *Scenedesmus obliquus* in palm oil mill effluent (POME) medium taken from aerobic pond and to know the lipid concentration produced by *S. obliquus*. In this research, *S. obliquus* was cultured in various POME concentration, those were 20, 60, 80, 90, 95, and 100% v/v, while the concentration of inoculum was 30% v/v, and fatty acid content was analysed by gas chromatography. The results revealed that growth rate was decrease with the increasing concentration of POME (0.3545, 0.1792, 0.1566, 0.1268, 0.1158, and 0.1008/day), dry biomass weight was increase with the increasing concentration of POME (0.40, 1.20, 1.30, 1.77, 2.28, and 2.45 g/L), and lipid content also tended to increase with the increasing concentration of POME (13.10%, 36.59%, 36.65%, 50.37%, 60.40%, and 64.98%). Oleic acid and behenic acid were the dominant fatty acid content in *S. obliquus* lipid cultured in 100% concentration POME media. It is concluded that aerobic-pond POME can be utilized as growth medium for *S. obliquus* with a high lipid content.

1. Introduction
Palm oil mill effluent (POME) is waste which still has high concentration of organic compounds and become one of potential pollutant to the environment [1]. In every ton of crude palm oil (CPO) production, it is needed 5 to 7.5 ton of water and more than 50% will end as liquid waste [2,3], and 0.5-0.75 ton of POME will be produced from every ton of palm fruit bunches [4]. POME is come from the processing of oil extraction, washing and cleaning, and also contain cellulose material, fat, and oil [5]. The high concentration of organic compound is valued as Chemical Oxygen Demand (COD). POME’s COD in anaerobic pond is ranged between 40,000 until 120,000 mg/L [5], whereas from aerobic pond is from 560 until 6,000 mg/L [6-8]. Although the COD level from aerobic pond has already decreased compared to the COD from anaerobic pond, but the waste has been not feasible yet to discharge into the river due to unfulfilled quality standard. Based on the regulation from the Ministry of Environment of Indonesia [9], the highest COD level from palm oil industry allowed to be discharged is 350 mg/L.

The high concentration of organic compound in POME waste can be utilized as nutrition for living organisms, such as bacteria, fungi, and microalgae [10,11]. POME utilization using living organisms will produce proteins [11,12], carbohydrates [12,13], nucleic acids [14], and also lipids [15,16]. Recently, lipid production is becoming a great concern, because it can be used as biofuel raw material. Public’s demand of fuel is increase whereas the availability of fossil fuel is decrease [17]. One of living organisms that can produce lipid in high number is microalgae. Researchers who have already used...
microalgae cultivated to produce lipid in high number and utilize it further for biofuel production are [18-20].

Researches using POME waste from anaerobic pond as microalgae growth media have already conducted by many researchers, but POME utilization from aerobic pond has never been conducted. Therefore, the objective of this research is to observe the utilization of POME from aerobic pond as *S. obliquus* growth media to induce lipid production in *S. obliquus*.

2. **Research methodology**

2.1. **Culture preparation**

Stock culture was prepared in 720 mL bold basal medium (BBM) with composition as in Toyub et al. [21]. BBM was autoclaved for 20 min. In 80 ml of *S. obliquus* in logarithmic phase was cultivated in BBM with pH 6-7, light intensity at 5,000 lux, and aeration. Culture was prepared in 1,050 mL BBM with addition of 450 mL of *S. obliquus* from stock culture. Culture was aerated and illuminated with 5,000 lux. Working culture was used for cultivation in POME.

2.2. **POME preparation**

As much as 2.6 L of POME was centrifuged at 3,000 rpm for 5 minutes to separate suspended solid from POME. After that, POME was diluted to obtain variation concentration of 20%, 60%, 80%, 90%, 95%, and 100% v/v with distilled water. Each concentration volume was 560 mL, and it was autoclaved for 20 minutes. POME pH was adjusted until 6.6, suitable condition for *S. obliquus* growth.

2.3. **Cultivation of Scenedesmus obliquus in POME**

*S. obliquus* cultivation in POME medium was conducted by adding 240 mL working culture into each variation concentration of POME. Culture growth condition is at pH 6-7, temperature 25°C, light intensity at 5,000 lux, and aeration. *S. obliquus* was cultured for 14 days. The cultivation was repeated three times.

2.4. **Biomass harvesting and lipid extraction**

Before harvesting, aeration was turned off and culture was left for 15 min until settled. Microalgae was taken in 7 of 50 mL-tubes, centrifuged at 3,000 rpm for 5 min. Supernatant was disposed, whereas the pellet was dried in oven for 24 h at 50°C. Dried biomass was weighed using analytical balance.

Two grams of dried biomass was mixed with 10 mL of methanol and 5 mL of chloroform, homogenized using vortex for 2 min and kept at 25°C for 24 h. Five mL of chloroform was added and the mixture was homogenized using vortex for 1 min. Mixture was added with 5 mL of distillate water and homogenized using vortex for 2 min. The formed layers were separated using separating funnel. Lipid at the lower layer was moved to 50 mL-tube as weighed-1 (*W*₁) and tube with lipid as weighed-2 (*W*₂). Lipid concentration was calculated using the following equation:

\[
\% \text{lipid concentration} = \frac{W_2 - W_1}{Dried \ Biomass \ Weight} \times 100\%
\]  

(1)

2.5. **Data analysis**

Dry biomass and lipid content were analyzed statistically using ANOVA with 95% confidence interval, whereas fatty acid content was analyzed using gas chromatography.

3. **Results and discussions**

Growth rate is a parameter that indicates microalgae ability to produce new cells by utilizing nutrition in its growth medium. Growth rate of *S. obliquus* in each concentration of POME is showed in table 1.
Table 1. Growth rate of S. obliquus in several concentration of POME.

| Medium  | Growth rate (µ) |
|---------|----------------|
| 20% POME | 0.3545/day     |
| 60% POME | 0.1792/day     |
| 80% POME | 0.1566/day     |
| 90% POME | 0.1268/day     |
| 95% POME | 0.1158/day     |
| 100% POME| 0.1008/day     |

One of the factors that affects the decrease growth rate of S. obliquus with the increasing POME concentration is the high suspended solid in POME media, especially in concentration 100%. Suspended solid affects the light penetration that is needed by microalgae for photosynthesis. In the research conducted by Sari et al., [22] about the effects of light intensity to Spirulina growth rate, indicated that microalgae growth rate in 20% POME media was higher than in 60% POME media. Lack of light intensity will inhibit cell growth in high concentration of cultivation media.

Beside light intensity, nutrition balance is also an important factor in microalgae growth. Microalgae need macronutrient such as carbon, nitrogen, hydrogen, sulfur, potassium, magnesium, and phosphorus that are used to produce biomass [23]. Concentration of 100% POME consisted of higher macronutrient such as carbon and nitrogen compared to 20% POME. The excess nutrition in 100% POME will decrease S. obliquus growth rate because the unused excess carbon and nitrogen for photosynthesis will settle and poison the S. obliquus. Although carbon can support microalgae growth and cell biomass production, but if it is in an excess number it will inhibit microalgae growth [24]. This phenomena was also happened in research conducted by Sari et al. [22]. Microalgae which was cultured in high concentration of POME revealed a decrease growth rate because of the excessive nutrition in POME media, therefore the unused nutrition will settle, and will poison and inhibit microalgae growth rate as well. On the other hand, Procházková et al. said that to overcome stress condition affected by the excessive nutrition in growth media, microalgae will change its metabolic pathway to produce lipid compounds as food reserves [25].

![Figure 1. Dry biomass concentration with the increasing concentration of POME media.](image1)

![Figure 2. Lipid concentration with the increasing concentration of POME media.](image2)

The profile of dry biomass weight produced by S. obliquus from each POME concentration is showed in Figure 1, whereas the profile of lipid concentration produced by S. obliquus from each POME concentration is showed in Figure 2. Correlation analysis between dry cell weight and lipid concentration resulted the value of 0.901. This value showed that dry cell weight and lipid concentration have a significant correlation, and positive correlation means that the higher the dry cell weight the higher the lipid concentration that it is consisted. Figure 1 showed that dry biomass concentration is
increase with the increasing concentration of POME, those are 0.40 g/L, 1.20 g/L, 1.30 g/L, 1.77 g/L, 2.28 g/L, and 2.46 g/L, respectively. Figure 2 showed the increasing of lipid concentration with the increasing concentration of POME, those are 13.10%, 36.59%, 36.65%, 50.37%, 60.40%, and 64.98%, respectively.

This result is supported by Show et al. [26] which cultivated Scenedesmus sp. in POME media taken from anaerobic pond with four concentration, those were 45%, 55%, 65%, and 75%. The highest dry cell concentration they gained was 1.47 g/L with lipid content 44.8% in 75% POME, whereas the lowest dry cell concentration was 0.86 g/L with lipid content 19.77% in 45% POME. The research conducted by Poh et al. [27] which utilized POME for culturing Scenedesmus dimorphus also supported the above results. With POME concentration of 20%, 60%, and 80%, the highest dry cell concentration was 0.77 g/L with lipid content 31% from 80% POME concentration, whereas the lowest dry cell concentration was 0.16 g/L with lipid content 3.42% from 20% POME concentration.

Lipid is a nonpolar compound with hydrophobic characteristic and soluble in organic solvent. The development units of lipid are fatty acids consisted of C4 until C24 of organic acids. Microalgae can produce lipids that content various organic acids. In cells, lipid functions as energy reserves in cytosol, citric acid will be converted into acetyl-CoA and then into malonyl-CoA, that will enter further to Kennedy cycle. Lipid in TAG form will be produced from Kennedy cycle.

Fatty acid content in S. obliquus lipid cultured in POME media was analyzed using gas chromatography, and the result is showed in table 2. The highest two fatty acid concentration are oleic acid (0.0239%) and behenic acid (0.0369%) produced by S. obliquus cultivated in 100% POME. This result is supported by the research conducted by Mata et al. [31]. In their research, lipid content in S. obliquus cultivated in synthetic media can be utilized as biofuel raw material because it consisted of several types of fatty acid such as myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, and behenic acid. The dominant fatty acids are oleic acid and palmitic acid with concentration of 21.59% and 47.8% w/w, respectively. If this result is compared to research conducted by Makulla [32], the types of fatty acid in S. obliquus lipid cultivated in synthetic media are myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and behenic acid, with the highest fatty acid concentration is behenic acid (59%). The other researchers that conducted lipid content research in S. obliquus cultivated in synthetic media are Abomohra et al. [33]. Their results showed that several fatty acids consisted in S. obliquus lipid are lauric acid, myristic acid, palmitic acid, stearic acid, and behenic acid, which was utilized as biofuel raw material, and the dominant fatty acid concentration is palmitic acid (15.71%).
Table 2. Types of fatty acid in S. obliquus lipid cultivated in POME media.

| POME concentration | Types of fatty acid | Fatty acid concentration (% w/w) |
|-------------------|---------------------|---------------------------------|
| 20%               | C14:0 Myristic      | 0.0061                          |
|                   | C18:0 Stearic       | 0.0062                          |
|                   | C22:0 Behenic       | 0.0059                          |
| 60%               | C14:0 Myristic      | 0.0065                          |
|                   | C18:1 Oleic         | 0.0069                          |
|                   | C22:0 Behenic       | 0.0057                          |
| 80%               | C14:0 Myristic      | 0.0063                          |
|                   | C18:1 Oleic         | 0.0079                          |
|                   | C22:0 Behenic       | 0.0036                          |
| 90%               | C14:0 Myristic      | 0.0064                          |
|                   | C18:1 Oleic         | 0.0170                          |
|                   | C12:0 Lauric        | 0.0067                          |
| 95%               | C18:1 Oleic         | 0.0115                          |
| 100%              | C18:1 Oleic         | 0.0239                          |
|                   | C22:0 Behenic       | 0.0369                          |

The similar types of fatty acid were obtained from other species of microalgae cultivated in POME media. Researcher Nur et al. [34] grew three species of microalgae, those were Dunaliella salina that produced palmitic acid (11.53%) as the highest concentration of fatty acid, Spirulina platensis that also produced palmitic acid (35.97%) as the highest concentration of fatty acid, and Chlorella vulgaris that produced linolenic acid (19.72%) as the highest concentration of fatty acid.

4. Conclusion
During cultivation, S. obliquus able to produce biomass along with lipid content enhancement. S. obliquus which was cultured in POME media has a potential to produce oleic acid and behenic acid, as the dominant fatty acids that can be utilized as biofuel raw material. Further research needs to be conducted to increase the concentration of dominant fatty acids by cultivating S. obliquus in undiluted POME media. Moreover, it is also needed to optimize growth condition supported factors to increase fatty acid production.

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