

# The influence of palm oil mill effluent (POME) pre-treatment as *Scenedesmus dimorphus* microalgae cultivation medium for biodiesel

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**Abstract.** Palm Oil Mill Effluent (POME) is a pollutant resulting from the processing of fresh palm bunches into crude palm oil. POME still contains nutrients that can be utilized for microalgae growth. The purpose of this research was to explore the influence of pre-treated POME using H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub> on its nutritional values. The pre-treated POME was applied on cultivation medium of *Scenedesmus dimorphus* microalgae. POME was pre-treated using H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub> with concentrations of 5, 10 and 30% (v/v). The pre-treated POME was added into a Bold Basal Medium (BBM). *S. dimorphus* was cultivated on the medium containing a mix of BBM and POME. Harvesting was executed in the stationary phase. Harvested *S. dimorphus* was dried, and lipids were extracted by using the Bligh & Dyer method. The transesterification was performed for the lipids and the results were examined using GC-MS. The GC-MS analysis showed that the fatty acid content in the extracted lipids was composed of saturated fatty acids and monounsaturated fatty acids. The fatty acids from *S. dimorphus* have the potential to become biodiesel.

**Key Words:** bold basal medium, extraction, GC-MS, microalgae, spectrophotometer UV-VIS.

**Introduction.** Currently, the majority of energy needs is fulfilled by utilizing fossil fuel. The extensive use of fossil fuel without providing alternative energy sources will cause the extinction of fossil fuel. Moreover, the combustion of fossil fuel causes gas emissions, such as CO<sub>2</sub>. These gas emissions are not environmental friendly, causing global warming and greenhouse effect. Because of that, the need for alternative energy sources friendly with the environment is inevitable. Currently, biodiesel is one of the alternative energy sources that is expected to substitute fossil fuel (Sari et al 2012; Amin et al 2017; Mahmud et al 2017). Biodiesel is a renewable and environmentally friendly energy source. These characteristics draw global attention to this alternative to fossil fuel. Biodiesel has identical physical and chemical characteristics with fossil fuel; thus, biodiesel can be used by conventional engines (Putra et al 2020; Saravanan et al 2020). Moreover, biodiesel has environmentally friendly emissions from combustion (Rajaeifar et al 2016). These days, biodiesel is already in the 3<sup>rd</sup> generation produced from microorganism lipids. Microalgae are some microorganisms that can produce lipids for biodiesel (Norfadilah et al 2016; Cheah et al 2018a; Shomal et al 2019).

Microalgae have become a promising biodiesel candidate because of their fast growth and high lipid production. Additionally, microalgae do not require vast areas to grow. Microalgae need a nutritious medium for growth. Bold Basal Medium (BBM) is one of the microalgae growth media, but it has high costs. Hence, it is needed to explore methods to lower BBM cost. Some industrial liquid wastes contain the nutrition needed by microalgae. Previous researches explored liquid waste from the processing of Fresh Palm Bunches (FPB) into Crude Palm Oil (CPO) as nutrition for microalgae. Cheah et al (2018b) cultivated *Chlorella sorokiniana* CY-1 microalgae using POME as a growth medium, and discovered that 30% (v/v) POME produce 11.21% lipid content in the algae (Cheah et al 2018c).

Indonesia is a country that produces a high volume of palm oil, with the palm oil industry growing every year. Along with the growth of the palm oil industry, the volume of POME also increases. POME requires further processing to avoid environmental polluting.  $H_2SO_4$  or  $HNO_3$  can hydrolysis POME and the result can be utilized for microalgae cultivation (Norfadilah et al 2016; Shomal et al 2019).

*Scenedesmus dimorphus* is a better microalga in lipid production compared to *Spirulina* sp., or *Chlorella sorokiniana* CY-1 (Cheah et al 2018a, 2018b). This research focused on POME hydrolysis using  $H_2SO_4$  or  $HNO_3$  to substitute BBM as a medium for microalgae cultivation. This research explored the different hydrolysis addition on the cultivation medium of *S. dimorphus* and analysed the lipid content of harvested *S. dimorphus*.

## Material and Method

**Tools.** This research utilized the following tools: cup glasses, centrifuge, autoclave, analytic scale (Metler), a spectrophotometer UV-VIS (Genesys 20), light microscope (Dynatech), Vortex Genie Pulse, GC-MS (Shimazu), water bath shaker.

**Materials.** This research used the following materials: POME, Bold Basal Medium (BBM), isolated *S. dimorphus* microalgae, aquadest,  $H_2SO_4$ ,  $HNO_3$ , methanol, hexane, chloroform, and NaOH.

**Sample collection and POME pre-treatment.** POME was collected from PT Incasi Raya, Pesisir Selatan, West Sumatera, Indonesia, and POME was pre-treated in the Biotechnology Laboratory of Andalas University, West Sumatera, Indonesia in 2020. POME was pre-treated using  $H_2SO_4$  1M or  $HNO_3$  1M. 94.4 mL POME was poured and stirred with 5.5 mL  $H_2SO_4$  1M, and 100 mL POME was poured and stirred with 6.95 mL  $HNO_3$  1M. The addition of  $H_2SO_4$  and  $HNO_3$  continued until the POME reach a pH of 1. Afterwards, it was filtered, and the filtrates were sterilized using an autoclave with a temperature set at 121°C for 15 min.

**Creating the medium for *S. dimorphus* microalgae cultivation.** The cultivation medium was created using different concentrations from the POME pre-treatment. The concentrations were 5, 10, and 30% (v/v). The medium was mixed with sterile BBM until the volume reached 100 mL. NaOH 1M was ass added into medium until the pH was 7-8. Afterwards, the cultivation medium was sterilized with a temperature of 121°C for 20 min (Zhang et al 2015).

**Cultivation of *S. dimorphus* and determination of its growth curve.** Isolated *S. dimorphus* were collected from the Biochemical Laboratories of Andalas University. They were cultivated on the medium with aeration by an aerator for 24 days. The lamp light was set to 21 watts with 12 hours of light and 12 hours of dark in the cultivation period. Purenness of *S. dimorphus* was identified using a light microscope at 400x zoom. The growth curve was determined by checking the time in day measurement versus an absorbance level at a wavelength of 680 nm ( $OD_{680}$ ).

**Harvesting *S. dimorphus*.** *S. dimorphus* were harvested at the final exponential phase or at the starting stationary phase. Harvesting was done by using a centrifuge at 3000 rpm for 15 minutes. Biomass was collected and transferred to a petri dish. It was aerated until it became dry (Cavonius et al 2014).

**Extraction and fatty acid analysis.** Lipid extraction was performed using the Bligh and Dyer method modified by Cavonius (Cheah et al 2018a). 20 mg of dry biomass were watered using 80  $\mu$ L aquadest for 1 hour. Afterwards, addition of methanol:chloroform (2:1) 300  $\mu$ L was performed and it was vortexed for 20 seconds. The mix was centrifuged at 2500 rpm for 5 min until chloroform, aquadest, and biomass phases were formed. The chloroform part was collected and transfer to a different container, then placed in an

oven with a temperature of 50°C for 30 min. Microalgae lipids were determined with the following formula (Cheah et al 2018b):

$$\% \text{ Lipid} = \frac{\text{Total Lipid Sample (gram)}}{\text{Weight of Biomass Sample (gram)}} \times 100\%$$

The transesterification was executed for each highest extracted lipid from every pre-treatment. The transesterification used methanol and H<sub>2</sub>SO<sub>4</sub>. After that, incubation was performed at a temperature of 90°C for 120 min. Hexane and aquadest were added and vortexed for 15 min. The hexane part was collected for fatty acid methyl ester analysis by using GC-MS.

## Results and Discussion

**The effect of POME pre-treatment and concentration on microalgae growth.** The purpose of POME pre-treatment was to hydrolyze the organic compounds in POME. The hydrolysis of organic compounds and acids can be additional nutrition for BBM, which is utilized in the *S. dimorphus* cultivation medium. A difference in POME pre-treatment concentration caused different growth of *S. dimorphus*. The growth of *S. dimorphus* microalgae cell was measured based on the absorbance at an optical density of 680 nm (OD<sub>680</sub>). The growth curve based on the different POME H<sub>2</sub>SO<sub>4</sub> pre-treatment concentration is presented in Figure 1.

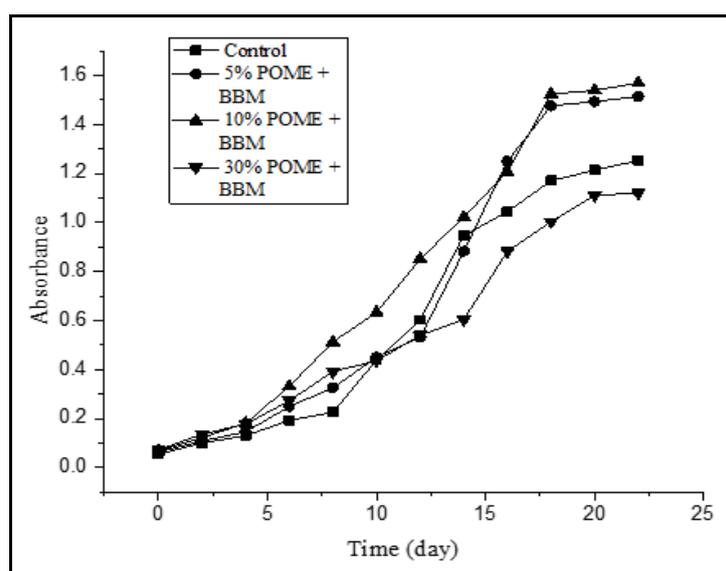


Figure 1. Growth curve of *Scenedesmus dimorphus* with different POME pre-treatment H<sub>2</sub>SO<sub>4</sub> concentration; POME - palm oil mill effluent; BBM - bold basal medium.

This research proved that POME pre-treatment with H<sub>2</sub>SO<sub>4</sub> increased the nutritional factors in the cultivation medium. The value of optical density (OD<sub>680</sub>) is high; thus, it can be concluded that there was an increment in the number of microalgae cells. POME pre-treatment with H<sub>2</sub>SO<sub>4</sub> converted lignocellulose into cellulose and glucose (Mahmod et al 2017). Cellulose and glucose were more convenient to be used as nutritional factors by *S. dimorphus* for growth. The 10% POME concentration resulted in the best growth of *S. dimorphus* compared with the other two concentrations. Light penetration was also better in the medium with the 10% POME. Light penetration helped *S. dimorphus* growth, as it is an energy source for cell metabolism (Cheah et al 2018a).

Figure 2 shows that 10% POME concentration has a higher absorbance value compared to the other two concentrations, thus being the optimum medium for growth among the three treatments.

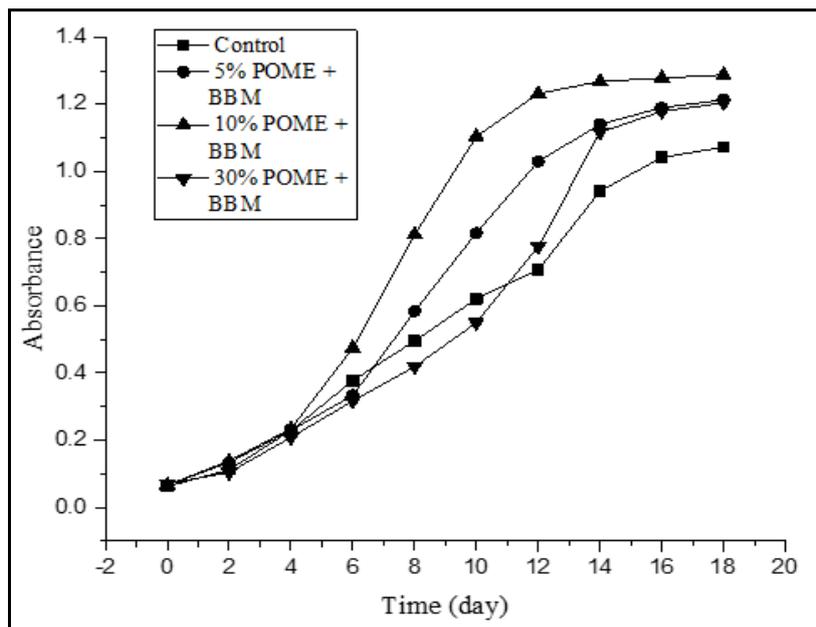


Figure 2. Growth curve of *Scenedesmus dimorphus* with different POME pre-treatment  $\text{HNO}_3$  concentrations; POME - palm oil mill effluent; BBM - bold basal medium.

**The effect of POME pre-treatment at different concentrations on lipid content.**

The harvesting of *S. dimorphus* was performed at the final exponential phase or at the start of the stationary phase of growth (Cheah et al 2018a; Ran et al 2019). All obtained biomass was dried, and its lipids were extracted. The highest lipid content was obtained when using 10% POME. Figure 3 shows the lipid content of *S. dimorphus* microalgae for each POME pre-treatment. The highest increment of total lipid content was obtained when using 10% POME. POME pre-treatment can increase the nutritional content in the cultivation medium. The higher carbon levels obtained from the lignocellulose hydrolysis helped increase lipid biosynthesis (Hadiyanto & Nur 2012; Amin et al 2017; Cheah et al 2018b).

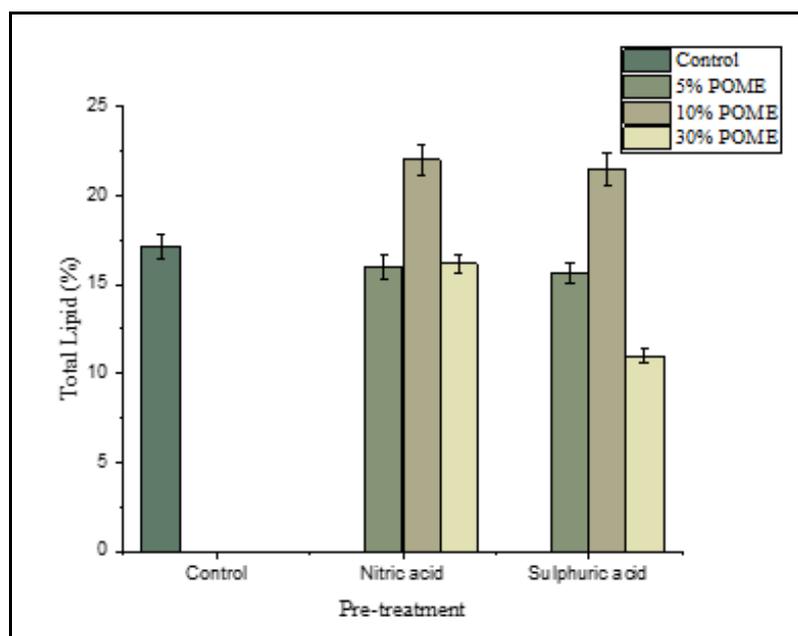


Figure 3. Total lipids of microalgae *Scenedesmus dimorphus* in various POME (palm oil mill effluent) pre-treatment.

**Fatty acid methyl ester analysis.** Fatty acid methyl esters were extracted from the *S. dimorphus* microalgae with the highest lipid content. The analysis results showed that the extracted fatty acid content could be utilized as biodiesel.

Table 1 shows the fatty acids in *S. dimorphus* lipids: palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). The fatty acids were saturated and monounsaturated. The composition of fatty acids influences biodiesel characteristics, such as oxidation stability, cetane number, melting point, and viscosity (Chuah et al 2016). One that shows the quality of biodiesel is that it contains polyunsaturated fatty acids and saturated fatty acids (Hadiyanto & Nur 2012), has the number of double bonds affecting biodiesel oxidation stability (Sharma et al 2015; Daneshvar et al 2018; Gao et al 2019).

Table 1

Fatty acid methyl esters profiles of microalgae *Scenedesmus dimorphus* cultivated in palm oil mill effluent media

Methyl ester	Fatty Acid	% Area		
		Control	H <sub>2</sub> SO <sub>4</sub> Pre-treatment	HNO <sub>3</sub> Pre-treatment
11- Methyl Hexadecenoic acid	Palmitic Acid (C <sub>16:0</sub> )	24.38	1.01	10.65
Methyl Octadecenoic acid	Stearic Acid (C <sub>18:0</sub> )	-	13.32	-
Tris[(Z)-2-methyl-9-octadecenoic acid]2-methylpropane-1,2,3-triyl ester	Oleic Acid (C <sub>18:1</sub> )	39.29	22.91	17.64

**Conclusions.** Based the results of this research, POME pre-treatment using H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub> could increase nutritional factors in BBM for *S. dimorphus* growth. The optimum concentration of POME pre-treatment was 10% (v/v). 10% POME addition would produce a high lipid content. POME pre-treatment using H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub> gained a lipid content value of 22%. Fatty acids obtained from *S. dimorphus* were palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). These fatty acids have the potential become biodiesel after further processing.

**Conflict of Interest.** The authors declare that there is no conflict of interest.

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Received: 12 February 2021. Accepted: 28 March 2021. Published online: 30 August 2021.

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How to cite this article:

Chaidir Z., Syaputra A., Mardiah E., Pardi H., 2021 The influence of palm oil mill effluent (POME) pre-treatment as *Scenedesmus dimorphus* microalgae cultivation medium for biodiesel. *AAFL Bioflux* 14(4):2487-2492.