International Journal of Engineering and Management Research e-ISSN: 2250-0758 | p-ISSN: 2394-6962
Volume- 9, Issue- 5 (October 2019)
www.ijemr.net https://doi.org/10.31033/ijemr.9.5.20

Utilization of Palm Oil Mill Effluent (POME) from the Residual Biogas Power Plant for Microalgae Production as Raw Material of Biodiesel

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ABSTRACT

Palm Oil Mill Effluent (POME) from the residue of biogas power plants generated by the Palm Oil Mill as the waste is still a problem in the processing environment and the environmental impact. However, POME waste still contains nutrients which are essential to the growth of microalgae as a growing medium. Utilization of POME can improve the growth of microalgae Botryococcus braunii compared to conventional media. By doing this project, the cost used by the palm oil mill waste management can be reduced and even be able to provide profits for palm oil mill and also can reduce the negative impact on the environment. The study was conducted at the Microalgae Laboratory of SBRC - IPB in September-Oktober 2018. Botryococcus braunii was cultivated in 1,000 ml erlenmeyer compared to standard medium as the control solution of 0.1% POME from the residue biogas power plants. Observation of Botryococcus braunii growth curve has carried out in accordance. Studies were arranged in CRD, with three replication. The results showed that the rate of Botryococcus braunii growth in POME is higher than the control for 2.7x10^6 sel/ml. And lipid extraction using osmotic shock method with HCl and CH_3COONa as osmotic agents in concentrations of 0.1; 0.3; 0.5 M, the highest yield of 0.108 g was obtained on the osmotic agent CH_3COONa 0.3 M.

Keywords— POME, Cultivated Microalgae, Lipid Extraction, Osmotic Shock

I. INTRODUCTION

Fossil energy is currently used for household, automotive, and industrial purposes, which has produced various kinds of gas pollution such as carbon dioxide, sulfur dioxide, smoke, fly ash, and so on, which causes global warming and climate change. One of the biggest producers of pollution and waste is from the palm oil processing industry

Palm Oil Mill Effluent (POME) is usually biologically treated in sewage ponds. Naturally, the treatment of wastes in reservoirs will produce methane gas (CH_4) and carbon dioxide (CO_2). Both of these gases are emissions that cause global warming, which is dangerous for the environment. During this time the two gases are allowed to evaporate into the air.

Palm oil liquid waste also contains acidic organic compounds as a result of microorganism processes. The results of the analysis of nutrient elements in palm oil liquid waste also showed the presence of nitrogen, phosphate, and mineral elements such as Na, K Mg, Fe, Zn, and Cu. Laboratory studies show that the pH value of palm oil liquid waste can be increased by adding base compounds. While the high content of organic matter can be reduced by using it first to produce biogas. The combination of the two methods can be utilized in an effort to produce microalgae biomass (Kawaroe, 2010)[1].

Electric power plants that use POME utilize methane gas (CH_4) into energy, which then leaves liquid waste that is still rich in nutrients and CO2 gas. The nutrients contained in the liquid waste can still be used as a source of nutrition for the cultivation of microalgae. Microalgae has the potential as a new alternative to renewable energy sources. Several species of microalgae are being developed in several countries as bio-oil producers, which can be further utilized in biodiesel.

Lipids in microalgae can be extracted directly without the drying process by destroying the microalgae cells (cell disruption method). One of the known cell disruption methods is the osmotic shock method (Mercer and Armenta, 2011)[2]. This osmotic shock method performs a sudden decrease in osmotic pressure in a microorganism that will cause cell damage. This method can be used to release cellular components such as oil.

Based on the research results, (Rachmaniah et al., 2010)[3]and(Yoo et al., 2012)[4]note that the use of osmotic agents with different types and concentrations results in lipid extraction will also be different. From the research of (Junaidi et al., 2014)[5] is known that the best osmotic agent is CH_3COONa, and (Rachmaniah et al., 2010)[3] the osmotic agent used was HCl. Based on this, the researcher is interested in examining the optimal type and concentration of osmotic agent for lipid extraction from Botryococcus braunii biomass with the osmotic shock method.

The purpose of this study was to examine the growth response of Botryococcus braunii microalgae

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produced from the cultivation media of POME wastewater from the remaining biogas power plant and obtain the optimal lipid extraction method by using osmotic shock extraction for the extraction of botryococcus braunii biomass as a raw material for biodiesel.

II. METHODOLOGY

The tools that will be used in this research are; photobioreactors made of acrylic and glass, plastic hoses, glass apparatus (dropper, erlenmeyer, test tubes, goblets and measuring cups), aerators, analytical scales, refractometers, thermometers, glass sample bottles, hemacytometers, incubators, aluminum foils, filters, oven, mortar, and spectrophotometer. And materials that will be used in this research are; microalgae starter Botryococcus braunii, POME liquid waste, Za fertilizer, urea, TSP, Guillard, liquid NPK, distilled water, alum, BF3 concentration 3%, NaOH, HCl, CH_3COONa and hexane.

Sterilization of Culture Tools and Media

Sterilization of tools and microalgae growth media is carried out before the microalgae culture stage at the laboratory and field scale. Sterilization is carried out with the aim to minimize the contamination of tools and culture materials when microalgae culture is carried out. Sterilization of laboratory equipment is done by referring to [1] used an autoclave with a temperature of 121ºC for 15 minutes of constant temperature, while sterilization of field scale media was carried out by adding 60 ppm chlorine / Ca (ClO) 2 within 24 hours and then adding 20 ppm of thiosulfate ($S_2O_3^{2-}$).

Propagation and Cultivation of Microalgae

Laboratory scale cultivation aims to reproduce microalgae culture with a volume of 1-5 liters. The ratio between liquid media and microalgae seeds is 1: 3. The media used is a mixture of water and POME with a ratio of 1: 1, according to (Hendroko et al., 2010)[6]. The results of culture at this stage will be used as a microalgae starter on a semi-mass scale.Semi-mass scale cultivation aims to increase the microalgae starter from the laboratory scale. In this cultivation, 50-100 liters of water media are used using technical fertilizers that refer to (Kawaroe et al,. 2015)[7].

Microalgae Lipid Extraction

Wet *botryococcus braunii* biomass as much as 10 g added 20 mL hexane and 50 mL solution (HCl and CH_3COONa) for the concentration level (0.1, 0.3, and 0.5 M). The extraction process is carried out for 24 hours, accompanied by stirring. The hexane layer was taken and then evaporated using a rotary vacuum evaporator to obtain microalgae lipids (Yoo et al., 2012)[4].

Esterification Process

Esterification aims to reduce the vapor point of fatty acids by changing the functional groups of fats into esters, which is relatively easy in GC-MS analysis. Saponified with 4.5 ml of 0.5 N NaOH, then put into a test tube and reacted with BF3 in methanol. Shake and heat for 1 5 minutes. Leave it to form two layers. The top layer is separated by centrifugation and further purification by adding Na2SO4 to removes the water content. The esterification results are then entered into vials to be analyzed with GC-MS tools (Hermanto and Muawanah, 2008)[8].

III. RESULT AND DISCUSSION

Botryococcus braunii microalgae density can be analyzed using a hemocytometer and microscope, and calculate it manually so that accuracy is maintained. Microalgae growth is obtained directly through the density calculator on the photobioreactor. Measurement of microalgae cell density starts from the day of first cultivation until the growth of microalgae dies. In this study, 1000 ml of freshwater media was used using standard media (30 ppm ZA, 30 ppm Urea, and 15% SP-36) as control and treatment by adding POME of the remaining biogas power plant as much as 0.1% from cultivation media.

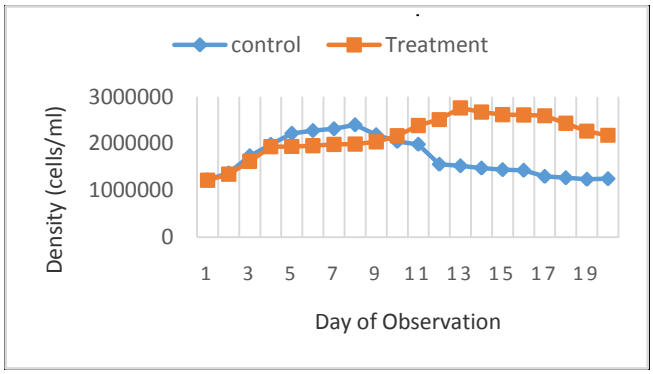

Figure 1: Growth cells density of microalgae *botryococcus braunii*

In the picture above, it can be seen that the addition of POME at the time of cultivation to replace standard fertilizers has a higher cell density, which reaches $2.7x10^6$ on the 13th day. Although the time to reach the stationary phase is quite long when compared to the control, the time for the stationary phase is longer before going to the death phase. While the highest cell density in the controlled media was $2.3x10^6$ on the 8th day, this is consistent with (Hendroko et al., 2010)[6]that the remaining biogas power plant can still be used as a microalgae growth nitration.

Biomass Lipid Extraction

Lipid extraction of microalgae Botryococcus braunii . By using the osmotic shock method, two osmotic agents were treated, namely HCl and CH_3COONa, with a

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concentration of 0.1; 0.3; 0.5 M, which can be seen in the following image.

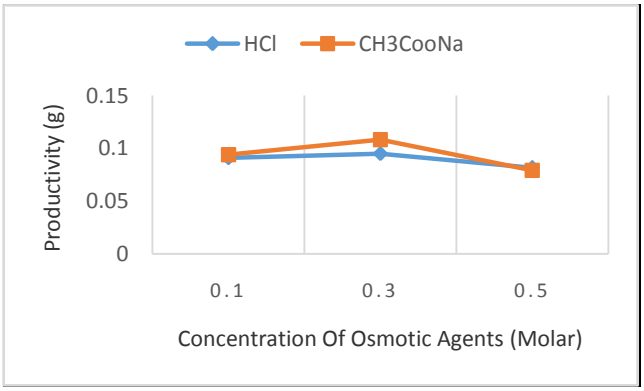

Figure 2: Graph of *Botryococcus braunii* lipid biomass extraction

From the picture above, we can see that extraction using osmotic agent CH_3COONa produces more lipids compared to HCl, and the concentration of CH_3COONa, which produces the most lipids, is a concentration of 0.3 M. According to (Yoo et al., 2012)[4], ionic osmotic agents are more effective at extracting lipids than non-ionic osmotic agents. This is because electrolyte solutions have a greater number of particles than non-electrolyte solutions at the same concentration, causing the osmotic pressure of electrolyte solutions to be greater when compared to non-electrolyte solutions (Dunlap et al., 2007)[9].

Esterification

Before esterification, saponification was carried out with alkali NaOH to form free fatty acids. After that, the esterification is done by reacting triglycerides with BF3 methanol to produce fatty acids methyl esters (biodiesel) with BF3 as a catalyst. The catalyst is used to increase the reaction rate and yield. This process takes place at 60 ° C with stirring using vortices, to increase the frequency of reactant collisions (Christie, 2012)[10]. This process is a two-way reaction, where triglycerides are gradually converted to diglycerides, and then methyl esters.

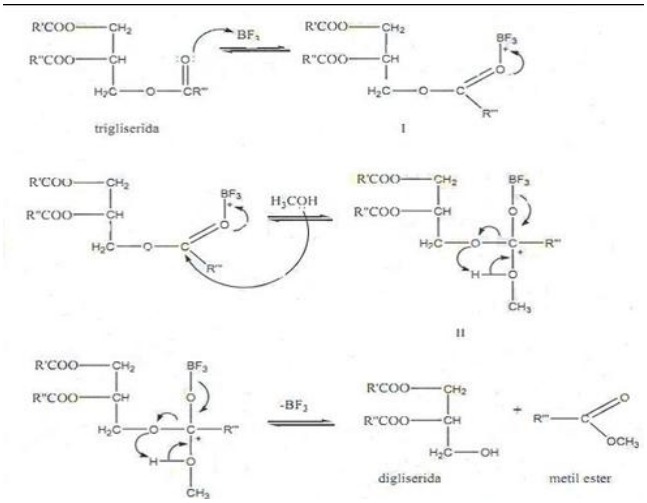

Figure 3: Esterification of triglycerides with BF3 methanol

Identification of Fatty Acid Methyl Esters of Microalgae

Identification of fatty acids methyl esters microalgae is made by looking at chromatograms of fatty acid methyl ester compounds that have been recorded for 30 minutes. The characteristics of the fatty acid methyl esters that appear in the mass spectra are characterized by a mass to charge ratio (m / z) 74. In addition, it is also seen based on the molecular peak, which shows the molecular weight value of the fatty acid methyl esters compound to determine the carbon number of the fatty acid methyl esters.

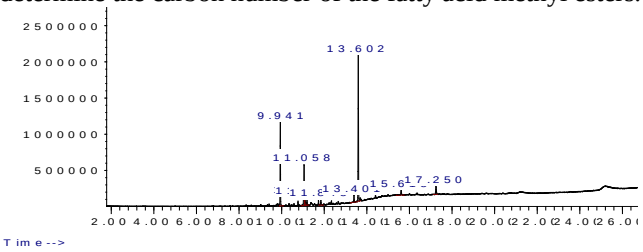

Figure 4: GC-MS results of methyl esters extracted using HCl as an osmotic agent

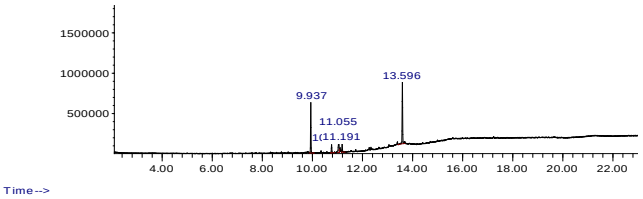

Figure 5: GC-MS results of methyl esters extracted using CH_3COONa as an osmotic agent

The types of compounds identified from methyl esters produced from the microalgae lipid Botryococcus braunii with GC-MS from the two osmotic agents used can be seen in the following table.

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TABLE I
The type of compound identified from both osmotic agents

	Compound	Total (%)
HCl	Metil Palmitate (C16:0)	22.173%
	Oleic acid (C18:1)	17.625%
	Metil octadecanoate (C18:0)	3.361%
	Metil Stearate (C18:0)	2.277%
	Metil behenate (C22:0)	2.138%
	methyl docosanoate (C22:0)	2.329%
	Benzenedicarboxylic acid	42.273%
	Methyl hexacosanoate (C26:0)	3.554%
	Methyl octacosanoate (C28:0)	4.270%
CH_3COONa	Metil palmitate (C16:0)	28.974%
	Pthalic Acid	5.230%
	Eleidic acid (trans-9 C18:1)	16.953%
	Metil Stearate (C18:0)	6.513%
	Benzenedicarboxylic acid	42.330%

According to (Borowitzka, 1988)[11], the major content of Bacillariophyceae (diatom) fatty acids consists of palmitic acid (C16: 0), hexadecenoic (C16: 1) and polynoic (C20), while the minor content in linoleic acid (C20). Triglycerides are produced by specific species/strains which are ultimately controlled by the genetic makeup of individual organisms. Microalgae produce small amounts of triglycerides under optimal growth or under favorable environmental conditions (Hu et al., 2008)[12]. Synthesis and high accumulation of triglycerides accompanied by considerable changes in the composition of fatty acids occur when microalgae experience stress conditions both in chemical and physical stimulation. The main chemical stimulus is nutrient impoverishment, while the main physical stimulus is temperature and light intensity. In addition, the growth phase of microalgae also affects triglycerides and microalgae fatty acid composition.

Effects of Fatty Acids Methyl Esters (FAME) on Biodiesel

The chemical composition of biodiesel and diesel fossils is very different. Diesel fossils usually consist of 30-35% aromatic hydrocarbons, 65-70% paraffin, and trace olefins, which are mostly in the C10 and C16 ranges whereas biodiesel contains C16 and C18 fatty acid methyl esters with one to three double bonds per molecule (Mittelbach and Remschmidt, 2006)[13]. Some parameters of biodiesel are influenced by lipids used as raw materials. The difference in density is influenced by the composition of fatty acids and the purity of raw materials. Density will increase with decreasing carbon chain length and increasing the number of double bonds in fatty acids, so the more unsaturated oil is used, the higher the density (Mittelbach and Remschmidt, 2006)[13]. As with density, the cetane number of biodiesel is also influenced by the composition of the fatty acid methyl ester, making up biodiesel. The more unsaturated fatty acid methyl esters contained in the oil, the lower the cetane number. That is what causes a decline in the quality of biodiesel. Based on the GC-MS results, it can be seen that Methyl Esters produced from extraction using HCl osmotic agents contain 40.102% more Saturated Faty Acid (SAFA) compared to methyl esters from the extraction results using 35.3487% CH_3COONa osmotic agent.

IV. CONCLUSION

1. Growth of Botryococcus braunii microalgae by using POME, the remaining biogas power plant as a nutrient produces a greater cell density than the control even though it prolongs the time to go to the stationary phase which is up to 13 days but extends the time for the stationary phase.
2. Lipid extraction results from Botryococcus braunii microalgae biomass. With the osmotic shock method and using the CH_3COONa osmotic agent at a concentration of 0.3 M has the highest productivity.
3. The GC-MS biodiesel test results showed that Methyl Esters produced from extraction using HCl osmotic agent contained 40.102% more Saturated Faty Acid (SAFA) compared to methyl esters from the extraction results using 35.3487% CH_3COONa osmotic agent.

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