Potential of Microalgae Oil Extract From Maluku Waters as a Renewable Fuel Source

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Abstract. Biodiesel is an alternative energy that is considered capable of answering the problem of scarcity of fuel oil. Potential sources of vegetable oil are microalgae which have higher oil productivity per unit of land area used when compared to land plants. The large potential of Microalgae is not widely known, especially those originating from the waters of Maluku, so research needs to be done to identify the types of microalgae and the quality of the biodiesel produced. Algae biomass was obtain by cultivation used Walne medium. The research steps include extracted microalgae oil with n-hexane, transesterification reaction of microalgae and methanol oil with BF₃CH₃OH catalyst, followed by filtration to separate biodiesel products with glycerol formed and tested viscosity based on ASTM D 445 method. The results obtained showed that the type of microalgae was Trichodesmium Erythraemia and had kinematic viscosity of 2.85 cSt. To determine the chemical content in raw materials and products, the extracted oil from microalgae and biodiesel produced ware analysed by GC-MS. From the resulted of the GC-MS test, it was found that the two largest fatty acid content in microalgae oil, namely Hexadecanoic acid at 13.19% which is FAME (Fatty Acid Methyl Ester), and 9-hexadecenoic acid (palmitoleic fatty acid) at 7.18% which is FAME (Fatty Acid Methyl Ester).

Keywords: Microalgae, Trichodesmium Erythraemia, biodiesel, Walne, cultivation, viscosity, GC-MS, Fatty Acid Methyl Ester (FAME)

1. Introduction

Maluku is one of the 34th provinces in Indonesia whose territory is adjacent to the tectonic plate that is close to the sea, so it is very potential to produce petroleum fuels, this is based on tectonic plate movement through tectonic earthquakes then trapped and suppressed sea water and everything in sea water including marine microalgae into
the soil to form an oil layer. In the laboratory, this can be demonstrated by squeezing microalgae cells to be able to push the oil out.

Bioenergy production as an alternative energy source is very effective in overcoming the problem of energy crisis while reducing environmental problems caused by the use of fossil energy sources. The Indonesian republic's government recently expressed its desire to support the development of alternative energy sources, including biofuels. This commitment was manifested in the presidential instruction No. 1/2006, to pursue the use and development of biofuels, including biodiesel from microalgae in Indonesia [1].

One type of renewable energy that is increasingly being used throughout the world is biodiesel. The name of biodiesel has been approved by the Department of Energy (DOE), Environmental Protection Agency (EPA) and American Society of Testing Materials (ASTM). Biodiesel is a biodegradable and non-toxic fuel that has a low emission profile, so it is not harmful to the environment compared to petroleum-based fuels.

Recent advances in bioresearch studies show that microalgae as single-celled photosynthetic organisms contain large amounts of fatty acids, which can be a very promising source of biodiesel. Microalgae is one of the organisms that can be considered ideal and has the potential to be used as raw material for biofuel production ([2]; [3]; [4]).

The lipid content in dry microalgae biomass of certain species can reach above 50% with very fast growth ([5]; [6]; [7]). The microalgae breeding process only takes about 10 days to be ready to be harvested so that mathematically its productivity reaches 120,000 kg of biodiesel / ha annually, its productivity is more than 20 times the productivity of palm oil which only reaches 5,800 kg of biodiesel per year and 80 times compared with 1,500 kg biodiesel / ha of castor oil every year [8].

Previous research shows that biodiesel production from microalgae per hectare is higher compared to oil from corn, soybean, sunflower, distance and oil palm which can only be developed in certain regions. In addition, microalgae have a rapid growth rate, and biomass can easily multiply within 24 hours even just 3 hours. Nurachman, et al., [1] has conducted research on the potential of tropical seas, namely benthic-diatom Navicula sp which is used as biodiesel. The growth profile was analysed by changing nutrient composition in three different media (Walne, ordinary sea water, and modified sea water) and light intensity. Navicula sp cells showed significant growth in Walne which was modified by sea water media. These microalgae grow well at a pH range of 7.8 - 8.4, and cells that are very sensitive to the intensity of direct sunlight exposure. ESI-IT-MS showed that triglycerides from algal oil were identified as POP (palmitate-oleat-palmitate), POO (palmitat-oleat-oleat), and OOLn (oleat-oleate-linoleic). Looking at the existing marine potential, it is very possible that biodiesel research from microalgae has been carried out by Nurachman, et al. Can be done and developed in eastern Indonesia, especially in Maluku Province.
2. Research Methods

2.1. Materials and Equipment

The materials to be used in this study are: chlorine solution, Na₂S₂O₃, KNO₃, Na₂HPO₄·12H₂O, CuCl₂·6H₂O, FeCl₃, HCl, Na₂SiO₃·5H₂O, urea, Vitamin B₁, Vitamin B₁₂, aquades, methanol, diethyl ether, hexane, 15 micron plankton net and microalgae samples. Equipment used: Soxhlet extraction, ESI-IT-MS instrument (HCT Bruker-Daltonic GmBH instrument), thin plate, analytic balance, pH meter, salinometer, glassware, fluorescent lamp (fluorescent lamp 216 olmol), a set of aeration tools and a set of SPSS data processing computers version 20.

2.2. Procedures

Microalga cultivation

The research was begin with microalgae cultivation. The first step was carried out without treatment of sea water which has the potential of microalgae from Maluku sea waters which have been filtered with 15 micron sized plankton net, cultured in space with temperature and lighting and controlled aeration for optimal growth of microalgae.

The microalgae stock was cultivate in a transparent glass bottle with a size of 9 cm in diameter, with a capacity of 1 litter which has been sterilized by soaking in chlorine solution for 24 hours. Next, neutralized with 45 ppm Na₂S₂O₃ and controlled lighting and aeration until inundated (approximately 8 days). The plankton seeds are inoculated into the medium that has been given 3 g urea with 200 mL of A solution which is 5 g of KNO₃ dissolved in 200 mL of distilled water; 500 mL of B solution is as much as 10 g of Na₂HPO₄·12H₂O which is mixed with 15 g of CaCl₂·6H₂O, 8 g of FeCl₃, 25 mL of HCl and dissolved in 400 mL of distilled water; and each vitamin B₁ and B₁₂ as much as 10 g, and 20 g, then aerated, then stored in a temperature room of 15-20 °C and in a place that is given a fluorescent lamp with a light intensity of 1,000 lux (20- 40 watt). After that the pellets obtainable used straining and approximately 80% for extraction, while the remaining 20% is cultivated again by adding sea water without the treatment want to be inoculated.

Biodiesel production from Microalgae

The results of microalgae biomass collected in the form of pellets were then extracted by lipids using diethyl ether and hexane. Lipids obtained from extraction of microalgae oil were separated and transesterified used methanol as well as sulphuric acid catalyst to produce methyl ester (biodiesel). Biodiesel formed can be separated using a separating funnel.

The characterization of microalgae biodiesel can be determined by measuring viscosity, density, acid number, iodine number, N-total, flash point analysis, cloud
point, sulphur and sediment content and strengthened by rapid number analysis using ESI-IT-MS. The study of biodiesel production was focused on the optimization of the microalgae lipid extraction process and transesterification process into methyl esters. Optimization of lipid extraction process was carried out on the type and concentration as well as the optimum time of lipid extracting solvent with parameters of lipid levels that can be extracted. Optimization of the transesterification process is carried out on the transesterification method; optimum time and temperature of the transesterification process with ESI-IT-MS parameters and the quality of biodiesel obtained.

2.3. Data Analysis

The data obtained were analysed based on the parameters to be examined as follows: Analysis of the oil content obtained from microalgae compared to the mass of pellets of algae harvested and multiplied by 100% so as to obtain the percent of oil content produced from microalgae.

3. Result and Discussion

3.1. Microalgae Identification

Microalgae seeds obtained from the waters of the village of Assilulu, Central Maluku Regency are taken conventionally with a depth of approximately 60 cm from the sea level, then cultured in a 3 litter’s volume of seawater using a photo bioreactor. Photo bioreactor is a tool for microalgae cultivation with a closed circulation system so that it can reduce the rate of evaporation of water and function as a tool used by microalgae to get sunlight so that the process of photosynthesis is optimal [9].

Photo bioreactor in the form of a transparent bottle, which has a volume capacity of 3.1 litters with a diameter of 20 cm. A very thin bottle wall can help light penetrate easily to the back of the reactor and avoid the occurrence of self-shading (the event of the closure of one cell by another cell which causes uneven light obtained by algae when the culture is getting denser), and equipped with an aerated hose connected with an air pump (air pump) with a capacity of 220V, 50 Hz; 3.5 L/min as many as 3 pieces, with the aim of supplying air and maintaining air circulation so that the algae do not settle on the basis of the photo bioreactor and remain homogeneous. This tool also uses two 21 watt fluorescent bus-light lamps that function as a substitute for sunlight in the microalgae photosynthesis process.

Culture was carry out at a temperature of 20-25 °C with continuous lighting intensity. The lighting process when culturing is divided into 3 parts, namely continuous lighting, dark light (photo periodicity) and lighting with increasing light intensity (alteration) [10]. This study uses continuous lighting with the aim of obtaining biomass in a short time, because microalgae cells continuously photosynthesize and produce new cells. The culture process is carried out for 8 days,
because the growth phase occurs which is characterized by changes in the colour of
water from colourless to green.

The identification process is carried out in order to determine the type of
microalgae. It was carry out using a Nikon eclipse 50i microscope with 400
magnification. The identification results showed that the microalgae obtained were
microalgae of Trichodesmium Erythraemia type which had long and pointed physical
characteristics, with a length of 188.13 - 239 µm and widths of 2.40 - 3.42 µm (figure
1). These microalgae are classified as Blue-Green algae (class Cyanophyceae) and are
usually found in shallow waters, tropical beaches, but in low density (Anderson, 1994).
Its presence in shallow waters and tropical beaches is due to the need for sunlight for
photosynthesis, especially red light absorbed from sunlight [11].

![Trichodesmium Erythraemia](image1.jpg)

Figure 1. The morphology of Trichodesmium Erythraemia (a), the size and diameter of
Trichodesmium Erythraemia with 400 times magnification used a Nikon eclipse
50i Microscope (b).

### 3.2. Microalgae Cultivation of Trichodesmium Erythraemia

The next stage of research was culture on laboratory scale Trichodesmium
Erythraemia. This process used the walne medium obtained from LIPI in the field of
Management and Dissemination of Maluku Research as nutrition. In order to stimulate
the acceleration of algae cell growth which will affect the amount of biomass
produced.

The Walne medium is used as a Trichodesmium Erythraemia culture medium
because it has a complete nutritional composition and in it there are macro compounds
that are very necessary for optimal growth processes in photo bioreactors such as
NaNO₃, CuSO₄, NaH₂PO₄, and CoCl₂ when compared to other media such as Benneck,
so that density higher cells and a longer growth phase. These was reinforce by
Andersen (2005) who said that cell growth will be influenced by the availability of the
main elements in the culture environment, namely in the form of C, H, O, N, P, K, S, Ca, Fe, Mg and the presence of micro nutrient elements (Mo, Cu, Zn, Fe, Si and Co). Components of vitamins available in the media can accelerate growth, especially vitamin B12 (Andersen, 2005). In addition, Walne used it as a medium in this study because Walne had been shown to be able to provide nutrients to blue-green microalgae in previous studies.

The observations at the time of cultivation showed an increase in the number of Trichodesmium Erythraemia cells from the first day to the fourteenth day. This can be seen when there is a change in sea water from colourless to thick green. According to Pelczar et al. [12]; Hoff and Snell, [13] this stage is called the growth phase which is referred to as the logarithmic phase or exponential phase because cell density occurs according to the time of culture where the growth rate is far greater than the death rate.

Then after the fourteenth day and on the next day there is a decrease in microalgae Trichodesmium Erythraemia cells. This stage is called the stationary phase where the growth rate becomes small and is proportional to the mortality rate. According to Metting and Pyne, [14]; Wijoseno, [15] decreases the number of cells due to reduced concentration of nutrients and reduced intensity of light received by Trichodesmium Erythraemia in consequence of the phenomenon of shadow formation (the phenomenon of self-shading) by microalgae cells in culture. The death phase occurs, when it was reaches the top of the population. These population reduction were due to the culture carried out on a limited volume which causes the amount of nutrients contained in the medium is also limited so that Trichodesmium Erythraemia is no longer able to maintain its density [16].

### 3.2. Biomasses

Harvesting is the process of separating between medium and microalgae in solid-liquid separation. This process were to separate the microalgae biomass contained in the reactor with the medium, so that biomass with a little water content is obtained [17]. Harvesting of microalgae biomass was often still an obstacle because not all biomass cultured can be harvested. These are makes microalgae harvesting very difficult and expensive [18]. Hulteberg et al., [19], suggested harvesting of the most efficient microalgae used chemical flocculants, because certain algae species have cell sizes <10μm. The use of chemical flocculants can precipitate biomass by as much as 80% [20]. Chemical flocculants can be used by adding pH to the harvest medium, for example the addition of potassium hydroxide which is able to add pH to reach 11 and sodium hydroxide to increase pH to 9 [19].

In this study, harvesting of Trichodesmium Erythraemia biomass was carried out by the method of flocculants, the deposition method using NaOH chemicals in a ratio of 1: 1 (1 L microalgae: 1 g NaOH) [21]. The flocculants culture was then flattened with an air pump in a bioreactor for 30 minutes. This is done so that the spread of flocculants is evenly distributed on the culture medium and this process is called
coagulation. After the coagulation process, the mixture of microalgae and flocculants is left for 4 hours until it is deposited at the bottom of the container. After that, the separation of biomass and clear liquid is carried out. The separated Biomass Trichodesmium Erythraemia was washed with distilled water several times with the aim of removing the salt content and biomass screening using a water-sucking cloth. In this method the results obtained are 350g of wet biomass / 12 Litter.

![Figure 2. Filtered biomass results of microalgae Trichodesmium Erythraemia](image)

3.3. Isolation of Lipid Microalgae Trichodesmium Erythraemia

The initial stage of making biodiesel from microalgae is the isolation of lipids from the dry biomass of microalgae Trichodesmium Erythraemia by 15 grams. Isolation using Soxhlet extraction method with extraction temperature of 65 °C for 24 hours using n-hexane solvent. In principle, extraction using Soxhlet is a continuous / continuous extraction because the solvent used to extract is always stable from the condensation results from solvent vapour [22]. The extraction results in the form of lipids dissolved in n-hexane were then separated by evaporation so that all n-hexane solvents were separated and pure lipids were obtained. The lipid weight of microalgae Trichodesmium Erythraemia was obtained at 1.01 grams with a lipid content of 6.73% from the dry weight of microalgae powder Trichodesmium Erythraemia. The lipid content obtained from the microalgae Trichodesmium Erythraemia did not reach 50% of dry biomass.

The synthesis of biodiesel from microalgae Trichodesmium Erythraemia was carried out by transesterification reaction using methanol solvent. Transesterification in vegetable oils is a reaction that occurs when triglycerides (fatty acid esters and glycerol) as vegetable oils, react with alcohol in the presence of a catalyst (acid or base) which produces fatty acid alkyl esters (other esters) and glycerol. The process of
transesterification used the microwave oven method, this method uses microwaves (microwave) which can propagate through fluids so that the heating process can take place more effectively and the process of making biodiesel can be done shorter [23]. This study used a boron trifluoride catalyst in methanol BF₃/CH₃OH. BF₃ was using as a catalyst because it was a Lewis acid which has a greater reactivity compared to mineral acids such as HCl, H₂SO₄, p-Toluene sulfonate and others, and produces a greater methyl ester rendamen. The microwave method is used to reduce energy and reaction time. After the fat transesterification process of the microalgae Trichodesmium Erythraemia was carried out used microwaves with the addition of 80 Watts of power in 1 minute reaction time using the microwave oven method, 2 layers were obtained. The top layer is a layer of turbid green biodiesel, while the lower layer is a layer of blackish brown glycerol.

3.4. GC-MS Results and Viscosity of Biodiesel Trichodesmium Erythraemia

Methyl esters from transesterification were tested using GC-MS to determine the quantity of compounds and their composition contained in the reaction results. This analysis of yields spectra peaks, each of which shows a specific type of methyl ester. GC-MS analysis results of the microalgae Trichodesmium Erythraemia methyl ester can be seen in figure 3.

![Figure 3. Biodiesel Chromatogram from Microalgae Trichodesmium Erythraemia](image)
Based on the chromatogram above, four biodiesel constituent compounds derived from microalgae were obtained as follows in the table 1;

Table 1. Methyl Ester in Biodiesel from Trichodesmium Erythraemia

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>% Compound</th>
<th>Molecule Formula</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>9,723</td>
<td>2.36</td>
<td>C₁₅H₃₀O₂</td>
<td>Methyl tetradecanoate</td>
</tr>
<tr>
<td>12,106</td>
<td>7.18</td>
<td>C₁₇H₃₂O₂</td>
<td>Methyl 9-hexadecanoate</td>
</tr>
<tr>
<td>12,337</td>
<td>13.19</td>
<td>C₁₇H₃₄O₂</td>
<td>Methyl hexadecanoate</td>
</tr>
<tr>
<td>14,460</td>
<td>4.05</td>
<td>C₁₉H₃₆O₂</td>
<td>Methyl 9-oktadecanoate</td>
</tr>
</tbody>
</table>

![Figure 4. (a). Methyl tetradecanoate ; (b). Methyl hexadecanoate](image)

![Figure 5. (a). Methyl 9-hexadecanoate; (b). Methyl 9-oktadecanoate](image)

**Viscosity Analysis**

Viscosity is one of the standards in biodiesel quality determine and has a very important to the process of injecting fuel. Viscosity that is too low can cause a fuel injection pump leak. The high and low viscosity values was the use of catalyst
concentration and temperature. It was because the excess catalyst concentration will accelerate the breakdown of triglycerides into three fat esters which will reduce the viscosity value by 5-10%. [24]. Kinematic viscosity obtained from the results was 2.85 cSt. When compared with the requirements of the Indonesian National Standard biodiesel quality from the Viscosity parameters recommended in ASTM D 445 of 2.3-6.0 cSt, then it meets the requirements.

4. Conclusion

To sum up, type of microalgae found in the Maluku waters was the microalgae Trichodesmium Erythraemia. It was has two main components biodiesel namely 13.19% Hexadecanoic acid which is FAME (Fatty Acid Methyl Ester), and 9-hexadecenoic acid compound (palmitoleic fatty acid) of 7.18% which is FAME (Fatty Acid Methyl Ester) respectively. Trichodesmium Erythraemia microalgae can be used as a biodiesel base material because it has a viscosity value of 2.85 cSt in accordance with the SNI-04-7182-2006 standard.

5. Acknowledgment

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