Biodiesel from microalgae *Nannochloropsis oculata* and *Tetraselmis chuii* by sonication technique and K$_2$CO$_3$ catalyst

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**Abstract.** The way out of the energy crisis is to divert national energy needs with the use of new and renewable energy. Bioenergy such as biodiesel is the expected one of those. Algae which has the potential to be used as raw material for making biodiesel is microalgae because it is easier in its cultivation. The microalgae used in this study were *Nannochloropsis oculata* and *Tetraselmis chuii*. Microalgae that have been harvested in the pasta form are extracted to obtain the oil then processed into biodiesel with an ultrasonic device. The transesterification reaction ran at 65°C with a 1% K$_2$CO$_3$ catalyst. The variables applied were the ratio of oil: methanol (1:5; 1:10; 1:15) and reaction time (1 and 3 hours). The biodiesel produced was then tested for acid numbers, %FFA, density, viscosity, and GC-MS analysis. The amount of *N. oculata* and *T. chuii* obtained from harvesting was 591.75 and 460.11 grams, respectively. The yield of oil extracted from *N. oculata* and *T. chuii* was 46.47% and 39.31%. Biodiesel yield obtained from *N. oculata* between 72.19 - 74.33% with the highest yield for N3 sample, meanwhile in *T. chuii* between 69 - 72.95% with the highest gain for T3 sample. In general, biodiesel samples meet national standard SNI for Biodiesel in density, viscosity and acid number. The methyl ester content of the GC-MS test in the best biodiesel samples is N1 sample at 68.4% and T6 sample at 79.49%.

1. Introduction

Increased energy consumption and decreased availability of energy sources that have occurred in the last decade, has the potential to cause fuel scarcity. The solution to overcome the energy crisis is to divert national energy needs by utilizing the availability of new and renewable energy (NRE). Bioenergy such as biodiesel is one of the expected NREs [1, 2]. The obstacle always faced is the availability of sustainable and non-edible raw materials. One of the raw materials that fit the category is algae [3-5].

Algae that have the potential to be used as raw materials are microalgae because they are easier to culture. Microalgae are believed to be able to provide a stock of energy-producing raw materials that are easily applied in life without the need for extensive productive land.

Research for the selection and culture of microalgae has been widely carried out by experts with the aim of producing oil as a substitute for fossil fuels with low operating costs. Much of the oil produced is selected from the type of microalgae and its culture process. Factors that influence it are light intensity, temperature, nutrients, CO$_2$, pH in the cultivation process and the method of harvesting it [6]. There are several microalgae harvesting techniques, i.e. centrifugation, filtration and
floculation techniques. Centrifugation and filtration techniques have the disadvantage of needing special conditions and expensive costs [7]. The technique of using floculants is to add what works to influence the pH value in the culture media so that microalgae can settle. Then microalgae deposition in culture is taken by filtering it using a small porous cloth.

The process of extracting and producing biodiesel is chosen using the sonication method for energy efficiency purposes. Ultrasonic waves are able to accelerate the reaction because the effects have a cavitation effect, heat effect, and structural effects that make the penetration of solutes into cells and homogenization can occur more quickly.

In the process of making biodiesel, the catalyst that is generally used is alkaline catalyst. The use of K2CO3 base catalyst [8, 9] for biodiesel synthesis is better than KOH. Alkaline catalysts such as NaOH and KOH are very sensitive to water content and free fatty acids. The presence of water can cause saponification of esters to form soap and cause emulsion formation. This situation is detrimental because the catalyst consumption increases and there are difficulties in the process of biodiesel refining.

Microalgae selected as alternative raw materials for biodiesel are Nannochloropsis oculata [10] and Tetraselmis chuii [11]. A few investigations of various microalgae states that the amount of lipid content in microalgae relies upon the strategy for development including restricting supplement admission, control of temperature and term of light power in the development medium [4, 12]. Growth varies on numerous components and can be improved for temperature, daylight usage, pH control, liquid mechanics, and more [13]. Both microalgae was selected based on data of lipid content, lipid production ability in the culture period and the types of microalgae species that already exist purely for culture. To find out the maximum lipid potential that can be obtained as an alternative raw material for producing biodiesel, it is necessary to conduct research to study it in the producing of biodiesel by sonication and K2CO3 catalyst assistance. From this research it is expected that both microalgae with all kinds of limited treatment given will produce optimum biodiesel with good quality.

2. Methodology

The systematic of this research consists of two stages; the first is preparation of raw materials including microalgae cultivation, harvesting and extraction processes; and the second includes the transesterification process, the analysis of biodiesel selected process results, and their comparison with biodiesel quality standards (Standard Nasional Indonesia, SNI).

The first microalgae cultivation was carried out in 10L gallons filled with 1L microalgae seedlings and 8L mineral water with 15g of mixed raw salt per 1L of water to form salinity conditions similar to brackish water. Nutrients given at 1 ml/L media culture in the form of a mixture of urea fertilizer 40 mg/L, ZA fertilizer 30 mg/L, and TSP fertilizer 10 mg/L. Provision of nutrients is done every 2 days. Culture media was replaced at harvest time on the day 14th of 18L media in a sterile 19L gallon by taking 2L microalgae on 9L media and the remaining 7L harvested. After the harvest period, the culture media was put into sterile 10L gallons by taking 1L of microalgae on the remaining 18L and 17L media were harvested.

Light intake of culture media uses 13w Philips LED in paired two pcs per gallon. Light exposure given under conditions of 18 hours of light and 6 hours of dark to obtain optimal growth results. For CO2 intake, a simple series of CO2 gas producers is made consisting of two 1.5L soda bottles. Each bottle contains a solution of baking soda and citric acid. The ratio of the solution is 1g of citric acid with 2 ml of water and 1g of baking soda with a ml of water. The solution of the citric acid in the pump by squeezing the body of the bottle slowly until the liquid of the citric acid rises through the hose and drips slightly into the bottle containing baking soda will react to produce pressurized CO2 gas. O2 intake obtained from aerators that flow through the hose. Tubes in the air supply and CO2 are fitted with bubble stones to reduce the gas bubbles that come out and enlarge their solutes into the culture media.

Harvesting is carried out by the method of floculation on every 14 days by adding alum.
Each addition of alum was done manually by stirring for 5 minutes and allowed to stand for 15 minutes until the top culture water deposition appeared clear. The culture media, which has formed the precipitate, are then copied into a bucket that has been covered with a cotton cloth to filter out the microalgal deposits. The microalgae that are held on the cotton cloth is then obtained in the form of a paste. The extraction process takes place in an ultrasonic device [14] with temperatures between 60–65\textdegree C and two hours long process and n-hexane solvent with the use of 10 ml for every gram of microalgae. Then the extraction results are transferred into the beaker by using filter paper to separate the pulp from the extracted solution. The extraction product is separated by the solvent using a rotary evaporator at a temperature of 70\textdegree C with a speed of 60-90 rpm for three hours.

The transesterification process is conducted in an ultrasonic bath with operating conditions of 65 \textdegree C and reaction time of 1 and 3 hours. Firstly, a solution of methanol is made in a beaker with the addition of a 1% by weight mass of catalyst K$_2$CO$_3$. Microalgae oil biomass is put into erlenmeyer as much as 15 ml per sample then placed in an ultrasonic device [14]. After that, the mixture of methanol catalyst is put into the erlenmeyer. After the process is complete, the product liquid is transferred into a measuring cup and then into the separating funnel. The liquid is allowed to stand for 24 hours so that the biodiesel solution forms a layer consisting of catalyst, glycerol and biodiesel. Furthermore, biodiesel will be analyzed for acid number, density, viscosity, \%yield and biodiesel composition using GC-MS. The results of the analysis obtained were compared with biodiesel standards.

3. Result and discussion

In the preliminary research, the total amount of biomass obtained from the harvesting of poles for 4 months was obtained, and the yield (\%) of oil extracted from the microalgae using n-hexane as follows (Table 1).

| Sample  | Mass (g) | Mass Product (g) | \% yield |
|---------|----------|------------------|---------|
| N. oculata | 591.750  | 275.003          | 46.470  |
| T. chuii  | 460.110  | 180.893          | 39.310  |

Generally, the percentage of extraction yield of N. oculata microalgae is higher than that of T. chuii microalgae. This is consistent with the productivity and lipid content with lipid content in Nannochloropsis sp. between 12 - 53\% of its dry weight and biomass productivity is around 0.17 - 1.43 g/L/day. While the lipid content in Tetraselmis sp. between 8.5 - 23\% of the dry weight of its time and its biomass productivity is around 0.12 - 0.32 g/L/day. Medipally et al reported from several sources that microalgae having different content of oil is able to produce oil with yield 30 – 70\% [15].

Biodiesel transesterification process with ultrasonic devices and K$_2$CO$_3$ catalyst have been tested for physical and chemical properties based on the treatment according to the research design with the following information:

- type of oil = produced from N. oculata microalgae and T. chuii microalgae
- reaction time = 1 and 3 hours
- oil: methanol = 1: 5, 1:10, and 1:15

Figure 1 shows the yield of biodiesel produced from N. oculata and T. chuii microalgae oil by varying oil-methanol ratio and reaction time. It is observed that the biodiesel yield reached in the range between 76 and 81\%. Generally, oil produced from N. oculata microalgae generates more biodiesel than that of from T. chuii microalgae. In addition, time of reaction affects negatively to yield. The change in reaction time from 1 to 3 hours decreases biodiesel yield, while the mole ratio of oil to methanol has a slight effect on yield. Maximum biodiesel yield is achieved by oil produced from N.
N. oculata microalgae with an hour reaction and 1:10 oil-methanol ratio. Nair et al. reported that algae biodiesel yield resulted over $\text{K}_2\text{CO}_3/\text{ZnO}$ was about 77% using 7% catalyst loading [16], while another also observed that it was over $\text{CuO}/\text{zeolite}$ with 3% loading was around 36.8% [17]. It was also reported that conversion of biodiesel from microalgae was about 87% over $\text{BaO}/\text{CaO-ZnO}$ catalyst [18]. A comparison of reaction conditions of those results is detailed in Table 2.

**Table 2.** The comparison of microalgae biodiesel performance over different catalysts.

| Microalga       | Catalyst System          | Catalyst Loading (%) | Yield (%) | Reaction time (hours) | Oil-methanol ratio (mole:mole) | Temperature (°C) | Ref.               |
|-----------------|--------------------------|----------------------|-----------|-----------------------|-------------------------------|------------------|-------------------|
| *Spirulina*     | $\text{K}_2\text{CO}_3/\text{ZnO}$ | 7                    | 77        | n.a.                  | 1:9                           | 80               | [16]              |
| *Chlorella*     | CuO/zeolite              | 3                    | 36.8      | 4                     | 1:4                           | 60               | [17]              |
| *S. platensis*  | $\text{BaO}/\text{CaO-ZnO}$ | 3                    | 87        | 2                     | 1:15                          | 65               | [18]              |
| *N. oculata*    | $\text{K}_2\text{CO}_3$  | 1                    | 81        | 1                     | 1:10                          | 65               | This research     |
| *T. chuii*      | $\text{K}_2\text{CO}_3$  | 1                    | 79        | 1                     | 1:10                          | 65               |                   |

Figure 2 presents the density measured at 40°C of microalgae biodiesel, and Table 3 is the quality standard of biodiesel based on SNI 7182:2015 in selected parameters according to this research. It is shown that almost all samples fall into the permissible quality range of biodiesel quality standards (0.85 - 0.89 g/ml). Only two samples do not meet the requirement in the biodiesel quality standard.

**Figure 2.** Density of microalgae biodiesel varying oil-methanol ratio and reaction time and produced from (a) *N. oculata* and (b) *T. chuii* microalgae oil.
Table 3. The quality standard of biodiesel according SNI 7182:2015

| Parameter                                | Value         |
|------------------------------------------|---------------|
| Density (40°C), gr/ml                    | 0.85 – 0.89   |
| Kinematic Viscosity (40°C), mm²/s        | 2.3 – 6       |
| Acid Number (max.), mg KOH/g             | 0.5           |

The kinematic viscosity data were measured at 40°C and Figure 3 exhibits this result. All samples are in the range of 3.20 – 3.25 mm²/s and meet the standard of SNI (Table 3). Figure 4 is bar charts showing the acid number all samples. It seems that only 4 of 12 samples of microalgae biodiesel prepared with 3 hours reaction fits the criteria according to SNI. The rest of samples shows higher acid number than the standard. This might be due to the microalgae oil contains high free fatty acid. It can be solved that the oil should be carried out esterification firstly before transesterification reaction.

Figure 3. Viscosity of microalgae biodiesel varying oil-methanol ratio and reaction time and produced from (a) N. oculata and (b) T. chuii microalgae oil.

In order to determine the composition of biodiesel, GC-MS measurement was performed using the Shimadzu GCMS-QP2010S instrument. Figure 5 shows the chromatograms of microalgae biodiesel sample. Table 4 lists the biodiesel components and composite ion. It shows that there are 32 peaks detected but only 8 peaks indicates methyl esters. The peak area containing methyl esters is about 79.5%. This result is in accordance with the calculation of acid numbers that shows the acid number is still high. The two major components are methyl palmitate and methyl 9-hexadecenoate.

Figure 4. Viscosity of microalgae biodiesel varying oil-methanol ratio and reaction time and produced from (a) N. oculata and (b) T. chuii microalgae oil.
Table 4. Component and normalized composition of microalgae biodiesel

| Component               | Composition (wt.%) |
|-------------------------|--------------------|
| methyl myristate        | 1.37               |
| methyl tetradecanoate   | 0.68               |
| methyl palmitate        | 61.73              |
| methyl palmitoleate     | 4.89               |
| methyl 13-decanoate     | 0.16               |
| methyl 11-octadecenoate | 0.92               |
| methyl heptanoate       | 0.24               |
| methyl 3-methoxy tetra decanoate | 0.94     |
| methyl 7,10,13-hexadecatrienoate | 0.70   |
| methyl linoleate        | 1.98               |
| methyl 9-hexadecenoate  | 16.79              |
| methyl stearate         | 1.70               |
| methyl eicosanoate      | 6.08               |
| methyl behenate         | 1.81               |

4. Conclusion
Lipid extraction has been successfully isolated from N. oculata and T. chuii microalgae and then synthesized into biodiesel using K$_2$CO$_3$ heterogeneous catalyst. A high microalgae biodiesel yield was observed during a one-hour reaction. Density and kinematic viscosity are mostly suitable with standard according to the SNI, however, the acid value is higher than the expectation. It can be concluded that N. oculata and T. chuii microalgae are potential resources for biodiesel production, although developments in the microalgae biodiesel processing field should be taken into account.
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