Potential utilization of *Botriococcus braunii* and *Nannochloropsis* microalgae as a biodiesel source in Indonesia

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**Abstract.** In recent years the ability of microalgae to accumulate triglycerides has motivated researchers to explore microalgae as a biodiesel source. Biodiesel is fuel from vegetable oils and animal fats that have properties resembling diesel oil. This study was aimed to determine the potential utilization of microalgae *Botriococcus braunii* and *Nannochloropsis* as raw material for biodiesel. Microalgae biomass with pH of 7 was extracted using supercritical CO\(_2\) devices. Microalgae biomass was treated with flowing liquid CO\(_2\) and a pressure of 90 psi at a temperature of 70°C. The super critical CO\(_2\) could extract 13.5% and 3% of oil from *Nannochloropsis* and *B. braunii*, respectively. Using GCMS, the oil from *Nannochloropsis* and *B. braunii* contained 22.23% and 15.78% of palmitic acid.

**Keywords:** biodiesel, *Botryococcus braunii*, microalgae, *Nannochloropsis*, supercritical CO\(_2\)

1. Introduction

At present, there is a strong dependency from human on fossil fuels such as gasoline, coal and natural gas. This is because more than 80% of the world's energy needs are derived from fossil fuels. But the problem with population growth is not in line with the domestic production of crude oil and its derivatives (Demirbas 2009). On the other hand, human realize that fossil fuels increase greenhouse emissions causing harmful effects on the environment and humanity. Thus, it is necessary to produce renewable fuels. Biodiesel has drawn attention in recent years because of its non-toxic nature, renewable, biodegradable and environmentally friendly (Li et al 2011). Microalgae are heterotrophic organisms that can be cultivated and can be used as a source for bioenergy. The biochemical composition of microalgae is very diverse (Satyanarayana et al 2011).

Microalgae is very interesting to be studied and developed as renewable fuel or biodiesel source. According to Christi (2007) microalgae is rich in lipids, however the amount of lipids depends on the type of microalgae, average growth and microalgae culture conditions. Microalgae can produce tri- and diglycerides, phospho-dan glycolipids, hydrocarbons, and others. Since the mid-70s microalgae have been broadly examined as a non-sustenance based biodiesel crude material because of its high oil content, fast development rates, high per hectare yields and the capacity to decrease grouping of
ozone-depleting substances in the air. Microalgae is generally more effective in photosynthesis and CO₂ fixation than land plants; 1 kg of algal biomass requires about 1.8 kg CO₂ (Sudhakar et al 2011).

*B. braunii* and *Nannochloropsis* are green microalgae from the living class Chlorophyceae. Chlorophyll content reaches 1.5-2.8% consisting of chlorophyll a, b, and c resulting yellowish green-brown colour. *B. braunii* is rich in hydrocarbons reaching 15-76% of dry weight. Long chain hydrocarbons of this species are known as botryococcin and very potential as an energy source or biodiesel (Panggabean 1998). *B. braunii* and *Nannochloropsis* reproduce very fast with short life cycle (Kabinawa 2008).

2. Materials and methods

2.1. Materials

The raw materials used in this work were *B. braunii* and *Nannochloropsis*. The equipments used were centrifuge, a supercritical fluid extractor and laboratory glassware.

2.2. Methods

2.2.1. Cultivation of *B. braunii* and *Nannochloropsis*. Starter cultivation of *B. braunii* and *Nannochloropsis* were done in the laboratory using seawater with salinity 25 ppt in a 30-liter container. The containers were placed at room temperature (25°C) added with fluorescent light at intensity 2000 lux. The results of laboratory-scale starter cultivation then spread on mass scale culture (outdoor) in a 1000 liter capacity container using salt water with 25 ppt, continuous aeration and sunlight for the process photosynthesis. Conwy fertilizer were used for cultivation.

2.2.2. Biomass collection. The harvested biomass was centrifuged for 30 minutes using a JA-14 rotor centrifuge with a speed of 10,000 ppm and a temperature of 4°C. The solution was removed and the supernatant was neutralized with distilled water and then centrifuged for 30 minutes using JA-14 rotor centrifuge at 10,000 ppm and 4°C. The collected supernatant was then ready to be extracted.

2.2.3. Extraction using supercritical CO₂. The extraction process was carried out using a supercritical fluid extractor which is equipped with the CO₂ gas cylinder, air compressor, high - pressure stainless steel extractor tube, extracted separator, heater, and chiller. The CO₂ gas was chilled in a chiller (at 5°C) prior to delivering into extractor at 5.5 mL/min. The temperature of the extractor was set at 70°C and the pressure of extractor was varied at 90 psi. At interval time of extraction, the extract was collected.

2.2.4. Analysis of fatty acid. Fatty acid profiles were tested based on the formation of fatty acid methyl esters using GC by a method developed by O’Fallon et al (2007).

3. Results and discussion

Supercritical CO₂ is a common approach to extract essential oils. This extraction process takes 4-7 hours. The results of oil extraction from the type of *Nannochloropsis* showed a value of 13.5±0.1%, while *B. braunii* of 3.00±0.34%. The process of collecting microalgae oil has a big challenge because microalgae biomass are still hard to collect on big scale because have very small sizes (generally 1-20 μm) and suspended in water. Several ways of harvesting microalgae are separating microalgae from suspension with sedimentation, flocculation, and centrifugation (Gerven 2009) however those methods require a lot of energy.

Esterification of microalgae oil was carried out to determine the types of fatty acids. The identification of oil using gas chromatography presented in table 1. It is known that palmitic acid as indicators of
biodiesel material from \textit{B. braunii} was 15.78\% and \textit{Nannochloropsis} was 22.23\%. It has been known that fatty acid profile affects fuel property. Saturated fatty acid will produce biodiesel with high oxidative stability and high cetane numbers, however due to low-temperature properties, this biodiesel tends to form a gel (Hu \textit{et al} 2008). Microalgae biodiesel must fulfill current standards, such as ASTM Biodiesel Standard D 6751 (USA) or Standard EN 14214 (European Union) meaning high cetane number is needed. Cetane numbers show the quality of ignition in the engine (Gerven 2009). Biodiesel with high unsaturated fatty acids has good flowability at low temperatures.

| Table 1. Fatty acid contents of microalgae \textit{Nannochloropsis} extracted by supercritical CO$_2$. |
|---------------------------------------------------------------|
| Carbon bond | Compound                                      | Percentage |
|--------------|-----------------------------------------------|------------|
| Saturated fatty acid |                                | 70.65\%   |
| 4            | Methyl Butyrate                              | 32.83\%   |
| 6            | Methyl Hexanoate                             | 5.04\%    |
| 16           | Methyl Palmitate                            | 23.24\%   |
| 24           | Methyl cis-5,8,11,14,17-Eicosapentanoic acid methyl ester | 9.54\%    |
| Unsaturated fatty acid |                            | 29.35\%   |
| 14:1         | Myristoleic acid methyl ester                | 2.79\%    |
| 16:1         | Methyl Palmitoleate                         | 8.36\%    |
| 18:1n9c      | Trans-9-Elaidic Methyl Esters               | 3.41\%    |
| 18:2n6c      | Linolelaiddic acid Methyl Ester             | 3.56\%    |
| 20:5n3       | cis-11,14,17-Eicosatrienoic acid methyl ester | 4.45\%    |
| 20:3n6       | cis-11,14-Eicosadienoic acid methyl ester    | 1.91\%    |
| 20:4n6       | cis-8,11,14-Eicosatrienoic acid methyl ester | 4.86\%    |

| Table 2. Fatty acid contents of microalgae \textit{B. braunii} extracted by supercritical CO$_2$. |
|---------------------------------------------------------------|
| Carbon bond | Compound                                      | Percentage |
|--------------|-----------------------------------------------|------------|
| Saturated fatty acid |                                | 68.36\%   |
| 4            | Methyl Butyrate                              | 13.16\%   |
| 6            | Methyl Hexanoate                             | 13.45\%   |
| 24           | Methyl cis-5,8,11,14,17-Eicosapentanoic acid methyl ester | 41.75\%   |
| Unsaturated fatty acid |                            | 31.64\%   |
| 16:1         | Methyl Palmitoleate                         | 5.15\%    |
| 18:1n9c      | Trans-9-Elaidic Methyl Esters               | 15.78\%   |
| 20:5n3       | cis-11,14,17-Eicosatrienoic acid methyl ester | 10.70\%   |

Based on the fatty acid content of microalga oils produced by supercritical CO$_2$, \textit{Nannochloropsis} and \textit{B. braunii} have potency producing biodiesel with good properties because it has high saturated fatty acid and unsaturated fatty acids. The biodiesel produced has high cetane numbers, high oxidative stability but still has the ability to flow at low temperatures.

4. Conclusion

Supercritical CO$_2$ produced low yield of oil suggesting that integration of cultivation, harvesting and research is needed to produce oil with high yield. Based on the results of fatty acid properties, \textit{Nannochloropsis} and \textit{B. braunii} have potency of producing biodiesel with good properties.
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