Fatty Acids From Microalgae Botryococcus braunii For Raw Material of Biodiesel

P Dilia¹, K Leila², Rusdianasari²

¹ Applied Master of Renewable Energy Engineering, Politeknik Negeri Sriwijaya, Jl. Srijaya Negara, 30139, Palembang, Indonesia
² Renewable Energy Engineering Study Program, Politeknik Negeri Sriwijaya, Jl. Srijaya Negara, 30139, Palembang, Indonesia

E-mail: puspadilia1602@gmail.com

Abstract. One of the alternative sources of renewable energy that can overcome the environmental crises and the current fossil energy crisis is microalgae. Microalgae contain fatty acids that can be converted into biofuel products, such as biodiesel. This study was a preliminary research which was conducted to determine the amount of fatty acids contained in microalgae and its potentiality to be converted into biodiesel. The microalgae used in this study were Botryococcus braunii. Data collection of fatty acids of Botryococcus braunii were obtained by the direct analysis in the laboratory using an instrument in the form of gas chromatography mass spectrometry (GC-MS) and also obtained from literature study in several research of previous researcher. Based on that, it was found out that Botryococcus braunii contained with oleic acid ranged from 15.65 – 35.85%, linoleic acid 3.05 – 23.15%, palmitic acid 4.03 – 22.13 % and stearic acid 0.48 – 15.41%. The profile and fatty acid content of this preliminary study could be one of the decisive parameters in the selection of microalgae species. Based on results concluded that Botryococcus braunii had good potentiality to be converted into biodiesel because the ratio of saturated and unsaturated fatty acids in this species is not much different.

1. Introduction
Over the last decade, the issue of environmental crises and the fossil energy crisis has become one of the global issues that motivates researchers in the world to conduct research concerning alternative and renewable energy. Oil-based biofuel derived from plants is one of the many solutions developed due to the availability of abundant raw materials and fairly simple production technology [1]. One research on biofuels that is being developed to be an alternative is to use photosynthetic microorganisms in the form of microalgae that are converted into biodiesel [2]. Microalgae, according to Mata et al. (2010), is a prokaryotic or eukaryotic photosynthetic microorganism whose growth is very productive and can outperform other land plants and is considered efficient enough to be used as biofuel due to the high fat or oil content and product of the photosynthesis from microalgae is also very high around 3 – 8%, while for land plants it only reaches 0.5% [3-4]. The potential comparison of some types of biodiesel raw materials can be seen in Table 1.

Table 1. Comparison of the Potential of Some Biodiesel Raw Materials [5]
One type of microalgae that can be utilized to biodiesel is Botryococcus braunii. Botryococcus braunii is a single green cell microalgae and is commonly found in the lakes, ponds, or brackish waters and the sea with a chlorophyll content of ± 1.5 – 2.8%. Botryococcus braunii has a cell nucleus with a size of ± 15 – 20 μm and lives in colonies. It is non-motile and each movement is strongly influenced by the flow of water. Botryococcus braunii is a potential source of renewable energy raw materials because this type of microalgae contains many hydrocarbon compounds. According to Samori et al. (2010), in the hydro cracking process of this type of microalgae, distillate can produce 67% gasoline, 15% aviation fuel, 15% diesel fuel and 3% residual oil. Fat content (fatty acids) of this type of microalgae is also quite high, ranging from 40 – 85%. In the process of photosynthesis, Botryococcus braunii also contributes significantly in reducing CO2 emissions, namely $1.5 \times 10^5$ tons y$^{-1}$ per $8.4 \times 10^5$ in the microalga cultivation area [6-8]. The compounds composition contained in Botryococcus braunii is shown in Table 2.

![Microscopic of Botryococcus braunii](image)

**Figure 1.** Microscopic of Botryococcus braunii [9]

| Raw Material        | Produced Lipid Yield (L/Ha) | Required Area Width (M ha) |
|---------------------|-----------------------------|----------------------------|
| Corn                | 172                         | 1,540                      |
| Soybean             | 446                         | 594                        |
| Canola flowers      | 1,190                       | 223                        |
| Jatropha curcas     | 1,892                       | 140                        |
| Coconut             | 2,689                       | 99                         |
| Oil palm            | 5,950                       | 45                         |
| Microalgae$^a$      | 136,900                     | 2                          |
| Microalgae$^b$      | 58,700                      | 4.5                        |

$^a$70% lipid assumption in biomass (% dry weight)

$^b$30% lipid assumption in biomass (% dry weight)

### Table 2. Compounds Composition in *Botryococcus braunii* [10]

| Compound Type | Composition (%) |
|---------------|-----------------|
| Chlorophyll a | 0.4             |
| Carotenoids   | 1.2             |
| Protein       | 17.8            |
| Carbohydrates | 18.9            |
| Lipid         | 61.4            |
Lipid are insoluble compounds in water of organic solvents such as chloroform hexane, toluene, and acetone, and lipids can also form combinations with other simple compounds such as triglycerides, and phospholipids [11]. Microalgae have a number of lipids with the same composition and even tend to be more than the other plants. The largest form of fat in microalgae cells is the triglycerides that can account for 80% of the total fat. This fat is the most important of the microalgae because it is the kind of oil that is good for producing biodiesel. Microalgae fats are usually esters composed of glycerol and fatty acids with a long chain of C14 to C22. The fatty acid composition of microalgae is monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), such as palmitic acid (C16:0), palmitoelic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), as well as several other types of acids [12].

The purpose of this study is as a preliminary research to determine the profile and composition of fatty acids contained in microalgae Botryococcus braunii and the relations between the identification of fatty acids and the potential of this type of microalgae as a source of renewable energy, i.e. biodiesel. Beside the direct research in laboratorium, researcher also adding another data as reference materials, some data obtained from some literature that discusses fatty acids Botryococcus braunii were implemented.

2. Material and Methods

The study was started with cultivation, extraction and transesterification process until biodiesel were obtained. The data for the cultivation and harvesting process of microalgae were obtained from direct observation conducted for ± 2 months in the laboratory of Process Unit of Chemical Engineering Department of Sriwijaya State Polytechnic, and the data of fatty acids were obtained by doing GC-MS analysis done in chemistry laboratory of materials processing of Bandung Institute of Technology. As supporting data of the main data obtained from the results of the study, the researcher used some literature study from some previous researchers. For the literature used as supporting data in this paper can be seen in Table 3.

| Ref. | Lead Author | Description |
|------|-------------|-------------|
| [8] | Rao         | Focused on the cultivation of B. braunii in two different media: raceway ponds and circular ponds from which both cultivation mediums obtained different composition of B. raunii fatty acids. |
| [13] | Ramaraj     | Evaluated the feasibility of biodiesel production directly from B. Braunii biomass at laboratory scale achieved through direct transesterification process. |
| [14] | Diraman     | Analyzed the fatty acid profiles of two types of microalgae namely B.braunii and P.cruentum from which it was known that B. braunii had potentiality as a source of biodiesel feedstock and P.cruentum was essential for fish feed. |
| [15] | Custódio    | Analyzed the profile and fatty acid composition of B.braunii and N. oculata from which it was found out that the fatty acid composition which was a biodiesel fatty acid in B.braunii was higher than N. oculata |

The study phase was begun with the cultivation of Botryococcus braunii microalgae seedlings obtained from the Balai Budidaya Perikanan dan Air Payau (BBPAP) in the area of Situbondo, East Java as much as 100 ml.
This microalgae cultivation was carried out in a culture bottle attached to the aerator with a duration of 10 days cultivation, and the cultivation medium used in the form of seawater with 25 – 35 ppt salinity of 500 ml. The medium of nutrition used in this cultivation is the walne’s medium as much as 1 ml on each cultivation. Lighting was done using a 20 watt TL lamp with a bright dark method with a 12 hour timeframe (12 light hours:12 hours of dark). Further harvesting and cell density calculations were performed using a hemocytometer on which the amount of the cell was calculated using equation (1) [16].

\[
\text{Number of cells per box} = \frac{\text{number of calculated cells}}{\text{number of boxes}} \times 25 \times 10^4
\]  

(1)

The second stage after cultivation was the process of taking lipids from microalgae that have been cultivated or known as the extraction process. Extraction method used was soxhlet method, that is extraction method which is done by heating and using solvent. The solvent used was 175 ml of n-hexane to extract 10 grams of *Botryococcus braunii*. This extraction process lasted for 4 hours in order to produce optimal lipids [16]. Furthermore, the obtained lipid was weighed and calculated in quantity by using equation (2).

\[
\% \text{yield} = \frac{\text{mass of extracted lipid}}{\text{initial sample mass}} \times 100\%
\]  

(2)

The last step conducted in this study was the making of biodiesel or FAME (Fatty Acid Methyl Ester) by using transesterification process with solvent in the form of methanol. The process was carried out for 2 hours until the two layers of triglycerides were formed on the bottom layer and FAME on the top layer. After that, several analyses of FAME were produced, one of which was fatty acid component analysis in FAME made from *Botryococcus braunii* by using GC-MS (Gas Chromatography-Mass Spectrometer) tool which was done in chemistry laboratory of material processing of Bandung Institute of Technology.

3. Results and Discussion

One of the parameters that could be considered as the reason in selecting the species of microalgae as the raw material of renewable energy, particularly biodiesel, is the lipid quantity resulted from the extraction process and the fatty acids composition owned by those microalgae. For this reason, this preliminary study was conducted in order to know the potential of *Botryococcus braunii* species of microalgae to be converted into biodiesel.

Results

This study was conducted through 3 stages, namely cultivation stage, extraction stage and FAME production stage. Then, several analyses and calculations were also done, the calculation of cell density amount, the calculation of resulted-lipid quantity, and the analysis of fatty acids. For the calculation of the amount of cell density and lipid quantity, the data were directly obtained from the conducted study. Meanwhile, for the analysis of fatty acids contained in *Botryococcus braunii*, the data were obtained from two sources which were the data of the study and the companion data from the existing literature (Table 3).

In the cultivation process done by the researchers, the value of cell density was observed by using microscope and hemocytometer chamber. The calculation of the amount of cell density was done in order to know the amount of microalgae cell developing during the growth period. By the duration of cultivation for 10 days, the highest amount of cell density was obtained on the 7th day of cultivation which was $5.208 \times 10^4$ cells/ml. The calculated data of the amount of the cultivated *Botryococcus braunii* cell density could be seen in Figure 2.
In the stage of extraction, the quantity of lipid rendemen, the extraction result, was calculated or % yield. % yield is a comparison between product mass and initial raw material mass [16]. The lipid rendemen resulted from extraction is colored as bright yellow and not too smelly and slightly thick. The lipid % yield resulted from soxhlet extraction was 24% with 175 ml of n-hexane solvent. After the extraction process was complete, the third stage, producing FAME, was done using transesterification method. The resulted FAME was bright yellow with a volume of ± 10 ml. From the resulted FAME, the composition of fatty acids from microalgae used as raw material that was *Botryococcus braunii* could be analyzed by using GC-MS instrument. Based on the results of the analysis, 3 types of fatty acid compounds in FAME produced, which was a compound contained in biodiesel, palmitic acid, stearic acid, oleic acid and linoleic acid, could be detected [12]. The graph of GC-MS analysis result from FAME and the composition of each fatty acid detected in the GC-MS analysis could be seen in Figure 3 and Table 4.

As a companion data, the researchers also conducted a literature study on *Botryococcus braunii* fatty acids from the previous studies. There were 4 literatures used as the companion data (Table 3). The

| Fatty Acid          | Retention Time | Area (%) | Height (%) |
|---------------------|----------------|----------|------------|
| Palmitic acid (C16:0) | 19.091         | 3.59     | 4.03       |
| Oleic acid (C18:1)  | 23.286         | 16.91    | 15.65      |
| Linoleic acid (C18:2) | 23.436        | 20.66    | 15.01      |

Table 4. Profile of *Botryococcus braunii* Fatty Acids in Direct Study
research data explained that there were 4 types of fatty acids contained in *Botryococcus braunii* that were biodiesel content. The composition of those four types of fatty acids in the studies could be seen in Table 5 and the comparison of fatty acid data from the results of direct study and previous study could be seen in Figure 4.

**Table 5.** Fatty Acids of *Botryococcus braunii* in Several Previous Studies

| Fatty Acid | Composition (%) | Ramaraj | Diraman | Rao<sup>a</sup> | Rao<sup>b</sup> | Custódio |
|-----------|-----------------|---------|---------|----------------|----------------|----------|
| C16:0     | 11.66           | 12.55   | 16.52   | 22.13          | 18.05          |
| C18:0     | 5.85            | 15.41   | 8.21    | 5.19           | 0.84           |
| C18:1     | 45.4            | 35.47   | 34.23   | 28.35          | 35.85          |
| C18:2     | 3.05            | 4.32    | 12.26   | 16.24          | 23.15          |

<sup>a</sup>cultivation in raceway ponds  
<sup>b</sup>cultivation in circular ponds

**Figure 4.** The Graph of *Botryococcus braunii* Fatty Acids Composition from Direct Study and Literature Study

Based on the existing fatty acids data, it was known that the range of fatty acid composition of *Botryococcus braunii*, especially for 4 types of fatty acid which was the biodiesel content was 4.03 – 22.13% for palmitic acid, 0.84 – 15.41% was stearic acid, then for oleic acid ranged between 15.65 – 45.4% and linoleic acid ranged from 3.05 – 23.15%.

**Discussion**

The growth phase of microalgae is generally divided into five phases of growth, namely lag phase, exponential phase, phase of growth rate lowering, stationary phase and phase of death [17]. From the research results, it was known that in the lag phase, the number of *Botryococcus braunii* cells were $2.257 \times 10^4$ cells/ml. Lag phase is an early phase of growth or adaptation of microalgae to the culture medium and nutrient medium used.

The highest growth phase of *Botryococcus braunii* occurred on the 7th day of cultivation with a cell number $5.028 \times 10^4$ cells/ml. This highest growth phase is called the exponential phase in which the cells divide rapidly and there is an increase in the number of cells. Based on the growth graph of *Botryococcus braunii* (Figure 2), it could be seen that the increase of *Botryococcus braunii* cells...
number occurred from the 2nd day until the 6th day of cultivation, yet the number of cells peaked on the 7th day. This happened because the microalgae cells cultivated have been in stable culture conditions.

After the peak increase or exponential phase, the microalgae cells would have the lowering of growth rate until there would be a stop of growth and the microalgae would undergo the phase with the lowest number of cells on the 10th day of cultivation. This phase is called as the death phase. The phase of death occurs as the stock of nutrients in the culture medium has decreased or discharged that causes microalgae cells in culture medium can no longer develop and slowly die until the number of cells in the culture medium becomes constant. The number of cells on the 10th day of cultivation was 2.604 × 10^8 cells/ml.

After the cultivation, microalgae were harvested and dried for later being extracted using the soxhlet method. Soxhlet extraction is usually used to extract the compound with limited solubility in a solvent and the impurities are insoluble in that solvent [18]. In this study, the compound of n-hexane was used as the solvent. The selection of this solvent was due to the nature of fat which is water-insoluble or polar solvent but soluble only in non-polar solvents and was also because the type of microalgae used in this study would be easily extracted by using n-hexane. The resulted lipid rendemen from soxhlet extraction using n-hexane of 175 ml had a yield % of 24%. The resulted rendemen was quite high considering that the sample of Botryococcus braunii extracted was only 10 grams. The volume of solvent used also affected the lipid produced with 175 ml of solvent that 45 solvent cycles were occured. Once the solvent is added then there will be more cycle happened and the resulted lipid rendemen will also increase.

Furthermore, the obtained lipid was converted to FAME, then the obtained FAME was analyzed to find out the profile and composition of the fatty acids contained there that whether the fatty acids of Botryococcus braunii was a component of biodiesel fatty acid or not could be identified. The analysis was performed using GC-MS instrument. From the analysis conducted directly, 3 types of fatty acids which were the components of biodiesel fatty acids, namely palmitic acid, oleic acid and linoleic acid were obtained, while the stearic acid was not detected by the instrument. This may be due to excessive sample storage time and poor storage mode allowing for the occurrence of oxidation in the sample before the sample arrived at the analysis site.

In the study conducted by Rao et al., (2012), two cultivations with different culture media, raceway ponds and circular ponds, in outdoor conditions were done. The total lipid produced by Botryococcus braunii was about 24% (w/w) with the dominant component of palmitic acid and oleic acid, both in cultivation using raceway and circular ponds [8]. The fatty acid profile contained in the microalgae oil was related to the biodiesel produced. Unsaturated fatty acids had a lower liquid point compared to saturated fatty acids that had good flowing ability at low temperatures. The opposite occured with saturated fatty acids that had a high melting point so that at low temperatures, it tends to not form a liquid or become a gel. According to Hu et al. (2008) and Chinnasamy et al. (2010), saturated fatty acids will tend to be at low temperatures but produce biodiesel with high oxidative stability [18-19].

According to Ramaraj et al. (2016), Botryococcus braunii is included to the microalgae which have good potential once being converted into biodiesel. It can be known from the high amount of lipid produced by Botryococcus and fatty acid profile owned by Botryococcus braunii was a component of biodiesel fatty acids as well. In the study conducted, it could be seen that the components of fatty acids dominantly owned by Botryococcus braunii were oleic acid, followed by palmitic acid, linoleic acid and stearic acid [13]. Lipids with high oleic acid content are known to have a good balance to the characteristic of fuel. The higher oleic acid in the fuel can increase oxidative stability for longer storage. Besides, Botryococcus has saturated and saturated fatty acids that are well balanced so that the biodiesel produced can still flow at low temperatures but have high oxidation values as well [13-14].

In a study conducted by Custódio et al. (2014) on fatty acids in Botryococcus braunii and Nannochloropsis oculata, it was also known that the fatty acid composition which was the content of biodiesel fatty acids in Botryococcus braunii was higher than the one in Nannochloropsis oculata and Botryococcus braunii was more potential to be converted to biodiesel compared to Nannochloropsis oculata for fatty acids in Nannochloropsis oculata were more dominated by saturated fatty acids [15].
4. Conclusion
Based on the study and literature study conducted, it could be known that *Botryococcus braunii* had a growth with a pretty high amount of cell density and could result plentiful lipid rendemen that had a good potential once being converted to biodiesel. Besides, some fatty acids components contained in *Botryococcus braunii* were the fatty acids components of biodiesel and the content of saturated and unsaturated fatty acids of *Botryococcus braunii* which were balanced that could be considered to be the raw material of biodiesel. The high composition of oleic acid in *Botryococcus braunii* could also be the basis in getting *Botryococcus braunii* as the raw material of biodiesel.

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