

Analysis of Lipid Yield from *Chaetoceros calcitrans* Microalgae Extract Using Gas Chromatography

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ABSTRACT

Renewable energy (RE) is a solution to the threat of the upcoming energy crisis. The increasingly massive energy transition is expected to significantly reduce the use of fossil fuels. Microalgae are one of the potential sources of RE for bioenergy (biodiesel) production due to their lipid content. This research was conducted to determine the percentage of lipid components; free fatty acids (FFA), monoglycerides (MAG), diglycerides (DAG), and triglycerides (TAG) in *Chaetoceros calcitrans* microalgae and to compare lipid yields using two extraction methods: Bligh-Dyer (wet samples) and Soxhlet extraction (dry samples). The results for *C. calcitrans* extracted using the Bligh-Dyer method showed that the percentage of lipid components (FFA, MAG, DAG, and TAG) ranged from 5.44% to 17.10%, with a total of 47.92%, and the lipid yield from 6 g of wet sample was 5.07%. Meanwhile, for the *C. calcitrans* extract using Soxhlet method, it was found that the percentage of lipid components (FFA, MAG, DAG and TAG) ranged from 6.79 to 18.01% with a total of 63.08% and the lipid yield from 20 g of dry samples was 0.44%.

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Introduction

The National Energy Policy, as outlined in Presidential Regulation No. 79 of 2014, targets a minimum share of renewable energy (RE) in Indonesia's energy mix of 23% by 2025 and 31% by 2050. This policy was developed in response to the global decline in fossil energy production [1][2] and to address the negative impacts of CO₂ emissions in Indonesia [3]. According to the Indonesia Energy Outlook 2019, the available RE potential in the country includes hydropower, geothermal, solar, wind, marine energy, and bioenergy [4], with microalgae being identified as one of the most promising sources of bioenergy [5].

Microalgae have significant potential as raw material for biofuel production (biodiesel, bioethanol, and biogas) [6] [7] due to their high content of lipids, proteins, and carbohydrates. Lipids can be converted into biodiesel through esterification and transesterification processes, while carbohydrates can be converted into bioethanol through fermentation [8]. The

potential of microalgae as a source of bioenergy lies in several advantages, including relatively easy cultivation, non-competition with major food crops, suitability for development in tropical regions with vast marine areas, and high production yields [9]. Another key advantage is their ability to reduce greenhouse gas emissions by absorbing CO₂ molecules during photosynthesis and converting them into biomass [10].

Chaetoceros calcitrans is one species of microalgae commonly found in Indonesian waters. According to Mata et al. [11], *C. calcitrans* has potential for biodiesel production due to its lipid content. Based on this background, this study aims to: (1) determine the percentage of lipid components; free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), and triacylglycerols (TAG) in *C. calcitrans* microalgae using Bligh-Dyer extraction (wet samples) and Soxhlet extraction

(dry samples); and (2) compare the lipid yields obtained from both extraction methods.

Materials and Methods

Materials

The materials used in this study included *C. calcitrans* microalgae samples in powdered and liquid/wet form (obtained from the Balai Budidaya Air Payau (BBAP) Situbondo), distilled water, aluminum foil, methanol, *n*-hexane, and chloroform.

Methods

The equipment used included laboratory glassware, glass vials, centrifuge, digital balance, oven, separatory funnel, Soxhlet apparatus, magnetic stirrer, rotary evaporator, and Gas Chromatography equipment (GC, Shimadzu GC-2010).

Moisture Content

Moisture content determination was carried out on wet microalgae samples. The microalgae biomass was separated from the liquid medium using centrifugation (speed: 500 rpm, duration: 4 minutes, at room temperature). The microalgae pellet that settled at the bottom layer was then weighed at 6 g and dried in an oven at 100°C. Drying was conducted until a constant weight (unchanging weight) was achieved, which was considered the dry weight of the microalgae. The procedure was performed in triplicate to calculate the average dry weight. The percentage of moisture content was calculated using Equation (1).

$$\text{Moisture content (\%)} = \frac{W - D}{W} \times 100\% \quad (1)$$

W : Wet weight of microalgae (g)

D : Dry weight of microalgae (g)

Bligh-Dyer Extraction Method

Extraction using the Bligh-Dyer method involves a combination of methanol-chloroform-water solvents [12] [13]. Wet microalgae samples were first centrifuged under the same conditions as in the moisture content determination stage (speed: 500 rpm, duration: 4 minutes, at room temperature). The microalgae pellet formed at the bottom layer was weighed at 6 g, then 30 mL of a methanol-

chloroform mixture (ratio 2:1, v/v) was added. The mixture was stirred for 15 minutes using a magnetic stirrer.

Next, 10 mL of chloroform and 10 mL of distilled water were added sequentially, with each addition followed by 15 minutes of stirring using the magnetic stirrer. The microalgae-solvent mixture was then transferred into a separatory funnel, shaken several times, and left to stand for 24 hours (three layers were observed in the funnel). After that, the chloroform layer (bottom layer), which contained the lipid extract, was separated. The chloroform solvent was then evaporated using a rotary evaporator to obtain the extract. The obtained extract was subsequently analyzed using GC. The percentage yield (wet basis and dry basis) was determined using Equations (2) and (3).

$$\text{Yield (wet basis, \%)} = \frac{E}{W} \times 100\% \quad (2)$$

$$\text{Yield (dry basis, \%)} = \frac{E}{D} \times 100\% \quad (3)$$

E : Weight of extract (g)

W : Wet weight of microalgae (g)

D : Dry weight of microalgae (g)

Soxhlet Extraction Method

The microalgae sample extracted using the Soxhlet method was in powdered form. The Soxhlet apparatus was first assembled, and then 20 g of microalgae powder was placed into the extraction chamber. A round-bottom flask was filled with 200 mL of *n*-hexane, and the heating mantle temperature was set to 69°C. The Soxhlet extraction process was carried out for 12 hours. After extraction, the *n*-hexane solvent was evaporated to obtain the extract. The obtained extract was then analyzed using GC. The extraction yield (dry basis) was calculated using Equation (4).

$$\text{Yield (Dry Basis, \%)} = \frac{E}{P} \times 100\% \quad (4)$$

E : Weight of extract (g)

P : Weight of microalgae powder (g)

Gas Chromatography Analysis

The extracts obtained from both extraction methods were analyzed using GC (Shimadzu GC-2010) equipped with a capillary column

(Phenomenex, 15 m × 0.32 mm × 0.1 µm) and a stationary phase of ZB-5HT (5% phenyl)-methylpolysiloxane. The injector temperature was set at 370°C. The oven temperature was programmed to start at 80°C, increase at a rate of 15°C/min to 365°C, and be held at 365°C for 8 minutes. Nitrogen was used as the carrier gas with a flow rate of 30 cm/s. A 1 µL volume of extract was injected. Lipid components (FFA, MAG, DAG, and TAG) were identified based on retention time comparison. Lipid yield (FFA / MAG / DAG / TAG) was calculated using Equation (5).

$$\text{Yield (\%)} = \frac{L \times E}{P \text{ or } D} \times 100\% \quad (5)$$

L : Lipid percentage, FFA / MAG / DAG / TAG (%)

E : Extract weight (g)

P : Microalgae powder weight - Soxhlet extraction (g)

D : Microalgae dry weight - Bligh Dyer method (g)

Results and Discussion

Moisture content

Table 1 contains the dry weight (g) and moisture content (%) values of *C. calcitrans*. The moisture content of *C. calcitrans* was determined to be 93.76%, indicating that this microalga has a relatively high water content. Previous studies have reported that the moisture content of microalgae typically ranges between 70–90% [14][15].

Table 1. Moisture Content (%)

Parameter	<i>C. calcitrans</i>
Dry weight	0,3743 g
Moisture content	93,76 %

Bligh-Dyer Extraction Results

Table 2 presents the results of the extraction using the Bligh-Dyer method. The obtained extract was dark brown in color and weighed 0.0396 g. This extraction method uses wet samples; therefore, the yield (wet basis, %) was calculated using Equation (2). The yield on a dry basis (%) was calculated using the dry weight obtained from the moisture content determination. This extraction method uses a combination of methanol–chloroform–distilled water solvents. Methanol (CH₃OH) and distilled water (H₂O) are polar solvents, while

chloroform (CHCl₃) is a semi-polar solvent. All three solvents have small molecular sizes, allowing them to diffuse more easily through the cell walls of *C. calcitrans*.

Table 2. Bligh-Dyer Extraction Results

Parameter	<i>C. calcitrans</i>
Extract color	Dark brown
Extract weight	0,0396 g
Extract yield (wet basis)	0,66 %
Extract yield (dry basis)	10,58 %

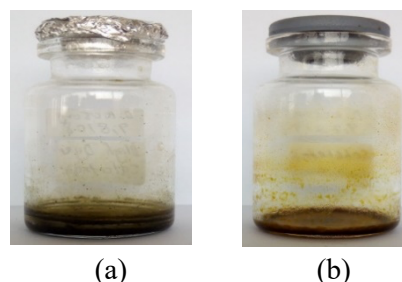


Figure 1. Extracts of *C. calcitrans* (a) Bligh-Dyer extraction (b) Soxhlet extraction

The purpose of stirring with a magnetic stirrer and shaking in a separatory funnel is to enhance the contact between the solvent and the microalgae, thus allowing more chemical components of *C. calcitrans* to be extracted.

Soxhlet Extraction Results

Table 3 presents the results of the Soxhlet extraction method. The extract obtained from the Soxhlet method was nearly four times greater in weight compared to that obtained by the Bligh and Dyer method. However, the yield (dry basis, %) from Soxhlet extraction was relatively lower, at only 0.69%. In the Soxhlet

Table 3. Soxhlet Extraction Results

Parameter	<i>C. calcitrans</i>
Extract color	Dark yellow
Extract weight	0,1382 g
Extract yield (dry basis)	0,69 %

method, a single solvent, *n*-hexane, a non-polar solvent was used. The purpose of using a non-polar solvent is to extract non-polar chemical components contained in *C. calcitrans*, primarily lipids (the target components). *n*-hexane is commonly employed in lipid extraction

processes due to its ability to produce relatively high extract yields, as well as its low cost [16][17].

Based on the comparison of the yield (dry basis, %) between the two methods, it is assumed that *C. calcitrans* contains a higher proportion of polar compounds than non-polar ones. The Soxhlet extraction process involves heating at 69°C (the boiling point of *n*-hexane) to convert the *n*-hexane in the round-bottom

flask from liquid to vapor. The vapor then rises and enters the extraction chamber, where it condenses back into the liquid phase upon cooling by the condenser.

Gas Chromatography Analysis Results

Figures 2 and 3 show the GC chromatograms of *C. calcitrans* extracts obtained using the Bligh-Dyer and Soxhlet extraction methods, respectively.

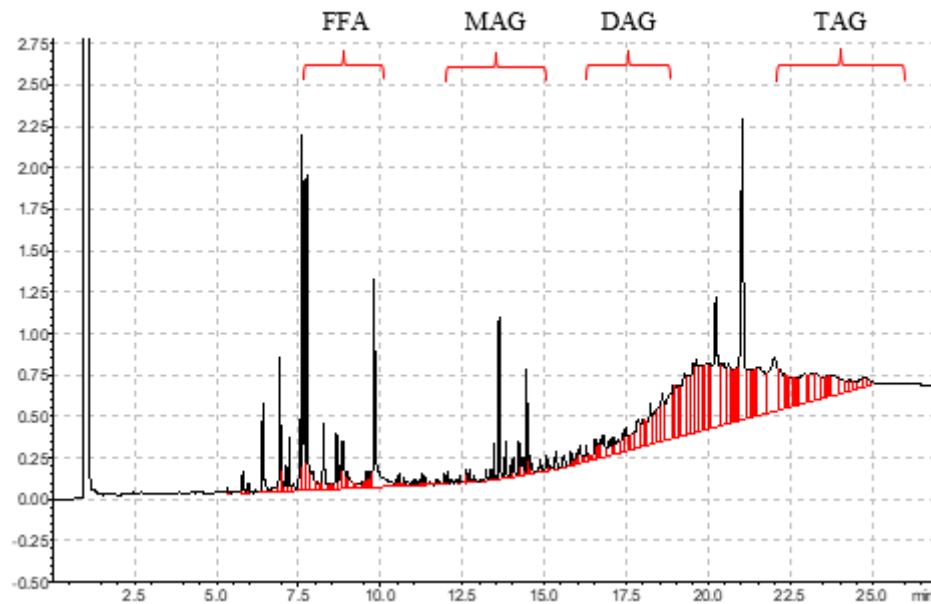


Figure 2. GC chromatogram of *C. calcitrans* extract using the Bligh-Dyer extraction method

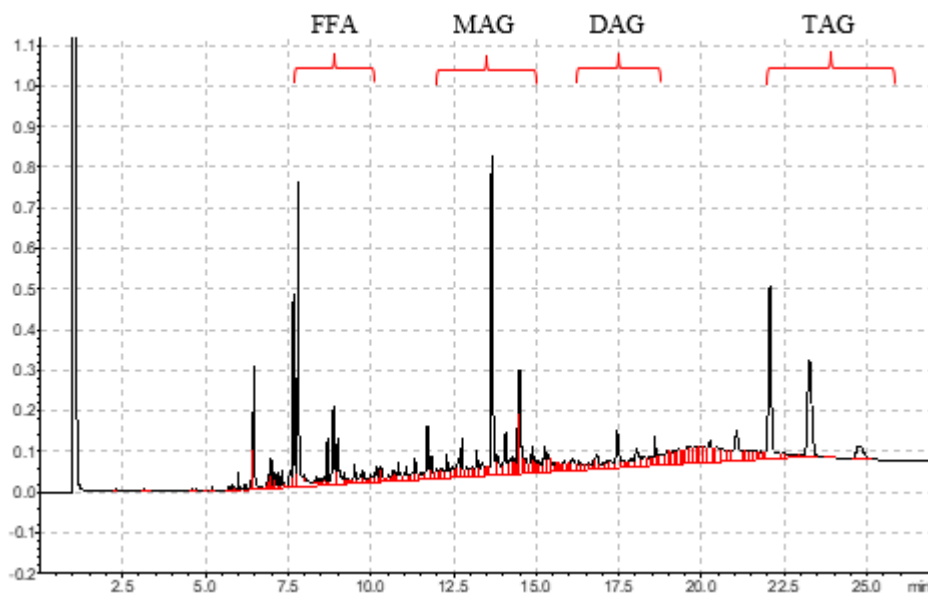


Figure 3. GC chromatogram of *C. calcitrans* extract using the Soxhlet extraction method

The GC chromatograms are used to analyze the percentage of lipid components present in the extracts. The lipid components include free fatty acids (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), and triacylglycerols (TAG) [18]. These four lipid components were detected at different retention times: 7.5–10 minutes for FFA, 12.25–15 minutes for MAG, 16.5–18.5 minutes for DAG, and 22–26 minutes for TAG.

Based on the GC chromatogram, the total lipid percentage from the Bligh-Dyer method was 47.92%, which is lower compared to the Soxhlet method. Table 4 shows that in the Bligh-Dyer method, the lowest lipid percentage was MAG at 5.44%, while the highest was TAG at 17.10%. In contrast, with the Soxhlet method, the lowest percentage was DAG at 6.79%, and the highest was MAG at 23.25%. Meanwhile, Table 5 presents the yield values for each lipid component (ratio to yield on a dry basis, %). The results from Tables 4 and 5 are subsequently

presented in graphical form in Figures 4 and 5, respectively. Based on the comparative graphs in these figures, it is evident that the lipid yields from the Bligh-Dyer method for all four components are higher than those from the Soxhlet method.

Table 4. Lipid Percentage Analysis Results

Extraction method	Lipid percentages (%)				
	FFA	MAG	DAG	TAG	Total
Bligh Dyer	16,57	5,44	8,81	17,10	47,92
Soxhlet	18,01	23,25	6,79	15,03	63,08

Table 5. Lipid Yield Percentage

Extraction method	Lipid yield(%)				
	FFA	MAG	DAG	TAG	Total
Bligh Dyer	1,75	0,58	0,93	1,81	5,07
Sokletasi	0,12	0,16	0,05	0,10	0,44

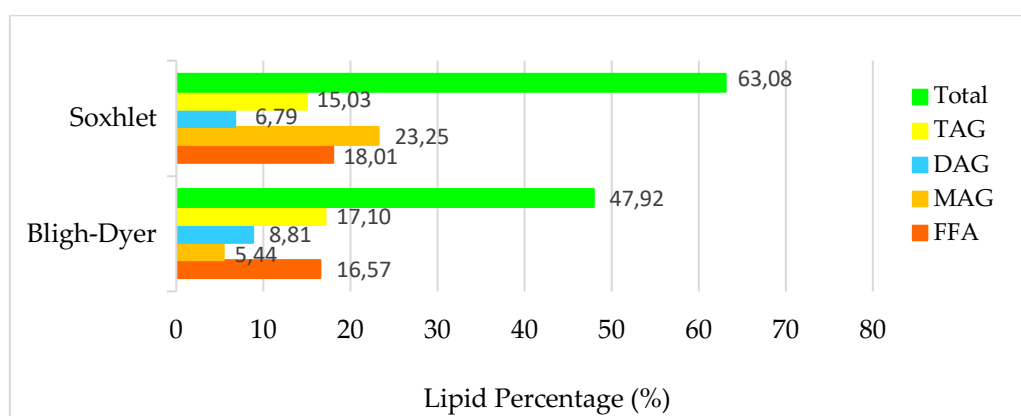


Figure 4. Comparison of the percentages (%) of FFA, MAG, DAG, and TAG from *C. calcitrans* extract using the Bligh-Dyer and Soxhlet extraction methods

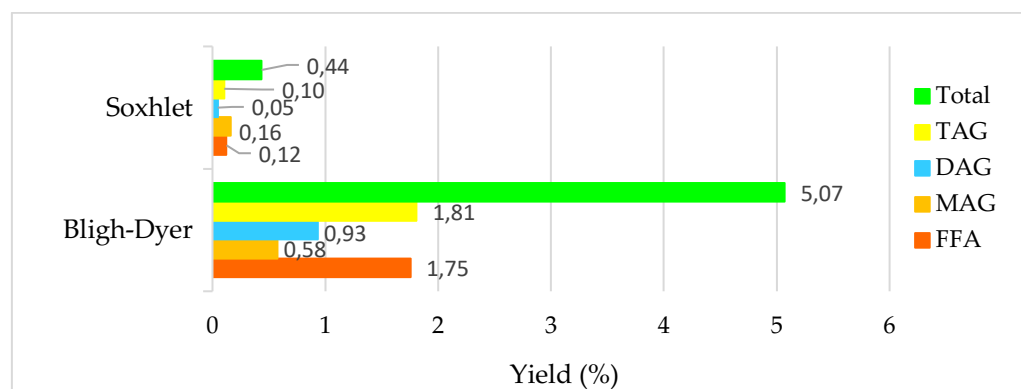


Figure 5. Comparison of the yields (%) of FFA, MAG, DAG, and TAG from *C. calcitrans* extract using the Bligh-Dyer and Soxhlet extraction methods

The lipid yield obtained from the Bligh-Dyer method was 5.07%, accounting for half of the total extract yield of 10.58%. Similarly, the Soxhlet method resulted in a lipid yield of 0.44%, which is more than half of its total extract yield of 0.69%. The use of magnetic stirring and shaking in a separatory funnel enhanced the contact between the solvent and the microalgae sample, resulting in a higher lipid yield from the Bligh-Dyer method compared to the Soxhlet method. One of the advantages of the Bligh-Dyer method is that it uses wet samples, thereby eliminating the need for a drying step during sample preparation [19][20][21]. Moreover, the Bligh-Dyer method does not involve heating during the extraction process, which helps minimize the degradation of extract components, particularly lipids compounds.

Conclusion

Based on the results of the study, the *C. calcitrans* extract obtained using the Bligh-Dyer method showed that the percentage of lipid components (FFA, MAG, DAG, and TAG) ranged from 5.44% to 17.10%, with a total of 47.92%. The lipid yield from 6 g of wet sample was 5.07%. In comparison, the *C. calcitrans* extract obtained using the Soxhlet method had lipid component percentages ranging from 6.79% to 18.01%, with a total of 63.08%, and a lipid yield of 0.44% from 20 g of dry sample.

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