Characterization and Rate Determination of Lipid Extraction from *Nannochloropsis* Using Stirring Method

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ABSTRACT

The rapid growth of the global population causes increasing energy needs. The energy needs currently used depend on non-renewable fossil fuels. An alternative to solve this problem is to use renewable fuel. One alternative to reduce the use of fossil fuels is microalgae because it contains lipids that can be processed into biodiesel. The microalgae used in this research is Nannochloropsis sp., which includes many lipids. This research was conducted to determine the total lipid content and percent extraction yield. The method used in this research is the mixing method. 7.5 grams of dried microalgae were mixed with a solvent mixture of 40 mL methanol and 90 mL hexane and then stirred at a stirring rate of 2000 rpm for 30 minutes, 60 minutes, 90 minutes, and 120 minutes. The most excellent total lipid content was obtained at 120 minutes; the amount received was 10.46%. The results show that in the extraction process, which lasted 120 minutes, most of the lipids were successfully extracted in the first stage, and the yield reached around 8.8% of the dry weight, or the equivalent of 84% of the total lipids present. Analysis of the extraction rate shows that the most optimal extraction process occurs in the first 30 minutes, where around 90% of the total lipids are successfully extracted. However, after the first 30 minutes, the rate of contraction tends to slow down. Based on these results, running the extraction process for 30 minutes is recommended to maximize yield.

1. Introduction

In recent years, the rapid growth of the global population has led to increased energy demand. This has resulted in the depletion of fossil fuel sources as the primary fuel [1], [2], [3]. The current energy demand relies solely on fossil fuels. Meanwhile, the natural sources of fossil fuels are limited and rapidly depleting, indicating that fossil fuels are not renewable as an energy source [4], [5]. In addition to these problems, the emissions caused can lead to global warming. An alternative to overcome these problems is using renewable fuels [5]. This solution not only solves energy problems but also environmental problems. This is because renewable energy sources come from plants, which absorb carbon dioxide for their growth, so carbon dioxide emissions from combustion are used to produce energy raw materials [6].

Renewable energy sources such as biodiesel are produced using vegetable oil as a raw material. However, vegetable oil is also a food source, so using it as a renewable energy source may lead to food security issues. This is a significant problem that needs to be addressed. This problem can be avoided by choosing non-edible vegetable oils or biomass waste as energy sources [7]. Several researchers have conducted several studies to obtain fuel from non-food and waste sources, such as biodiesel production using eggshell-based heterogeneous base catalysts [8], coconut fatty acid

distillate processed into biodiesel [9], gas production from bagasse [10], bio-oil production from wood, fruit and vegetable waste [11], biodiesel from *kesambi* seeds [12]. Recently, several researchers have investigated the production of third-generation biodiesel from microalgal lipids [7].

One alternative to reduce the use of fossil fuels and food security is microalgae [13]. Microalgae are photosynthetic protists, including prokaryotic cyanobacteria and eukaryotes such as green algae, and grow mainly in freshwater and seawater [14], [15]. According to statistics, 40,000 microalgae contain many macro and micro metabolites in nature, including proteins, carbohydrates, lipids, phenols, and minerals. The bioactive compounds in microalgae have been proven to be utilized in medicine, food, bioenergy, and other fields [16], [17].

In addition to their high content of bioactive compounds, microalgae have other advantages that allow them to be widely utilized: a) microalgae grow in water, helping to conserve land currently used for the agricultural industry and reduce land pressure; b) compared to other plants, microalgae grow and reproduce very quickly and can survive in harsher conditions; c) In agricultural production, microalgae can also be used to improve soil fertility by enhancing soil nutrient cycling, thus playing a positive role in plant growth; d) microalgae can reduce global warming because the photosynthetic process uses carbon dioxide. Besides oil, bio-oil production from microalgae biomass waste is another added value of utilizing microalgae as an energy source [18], [19], [20]. Overall, microalgae have great potential for future development and utilization.

The microalgae used in this study is *Nannochloropsis sp.* Because it is rich in lipids, it has the potential to be used as a raw material for biodiesel production [21], [22]. As much as 42.315% of fatty acids in *Nannochloropsis* are palmitoleic acid. According to the results of research conducted by [23], the fatty acid content of *Nannochloropsis sp.* contains caprylic acid (0.30%), lauric acid (0.99%), myristic acid (7.06%), palmitic acid (23.07%), oleic acid (12.25%), palmitoleic acid (42.32%) and linoleic acid (2.47%). In addition, according to [5], the lipid content of microalgae type *Nannochloropsis sp.* is 12-53%. The content of local and foreign microalgae is influenced by how the cultivation is carried out. Local microalgae usually come from native Indonesian strains found in fresh, brackish, and marine waters [24]. At the same time, foreign microalgae can come from strains imported from abroad. In addition, the resources used in local and foreign microalgae cultivation are also different. Local microalgae cultivation relies more on natural resources available around the cultivation site, such as water and sunlight. At the same time, foreign microalgae cultivation relies more on advanced technology and artificial resources, such as photobioreactors and synthetic fertilizers [25].

Producing biodiesel from microalgae involves extracting lipids from microalgae and then converting them into biodiesel through transesterification. There are several commonly used microalgae extraction methods, including extraction by stirring, Microwave Assisted Extraction (MAE) using microwave to accelerate the reaction, Ultrasonic Assisted Extraction (UAE) cell destruction, hydrodynamic cavitation, and so on [26], [27]. The method chosen in this research was stirring at a rate of 2000 rpm because it is the simplest method and uses tools that are easy to obtain and operate. However, this method is considered less efficient because the microalgae lipid extraction process takes quite a long time. An alternative that can be done to increase extraction efficiency is to choose the proper solvent, for example, by combining polar and non-polar solvents. This study aimed to characterize microalgae lipids extraction. This research contribution is to provide evaluation on the extraction rate based on the yield produced by the microalgae *Nannochloropsis sp*. using the stirring extraction method.

2. Research methodology

2.1. Research Materials and Tools

The raw material used in this research was microalgae *Nannochloropsis sp*. in dry form with a particle size of around 2-4 μm, obtained from the Brackish Water Cultivation Center in Situbondo, East Java, Indonesia. The solvents used are technical methanol and hexane obtained from CV. General Labora in Yogyakarta. All chemicals used do not undergo additional processing or purification. The tools used in this research include a series of extraction stirrers consisting of a hot plate with a magnetic stirrer and Erlenmeyer and tools for separating the extraction results, such as a centrifuge, distillation equipment, and analytical scales.

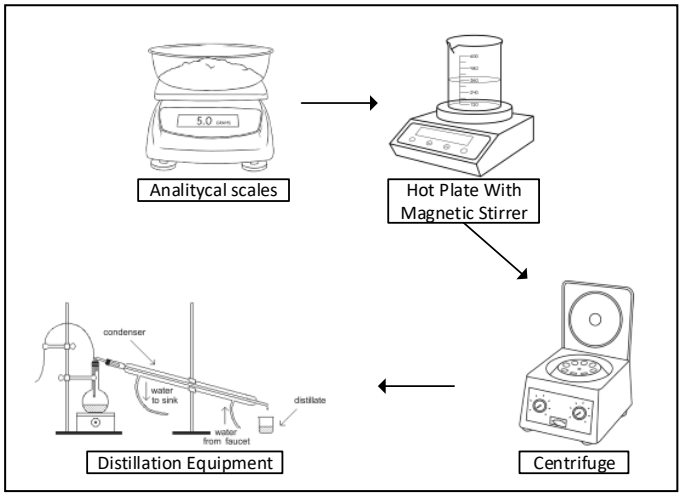

Fig. 1. Schematic diagram of the apparatus used in the experiment

2.2. Procedures

The study was conducted by extracting 7.5 gr of dried microalgae with a mixed solvent of 40 mL methanol and 90 mL n-hexane in an Erlenmeyer with a time variation of 30, 60, 90, and 120 minutes. After the extraction runs, the results were separated between solids and liquids by centrifugation at 2000 rpm for 5 minutes. To determine the repetition variable, repeat the same sample for 1 and 2 repetitions. For time variables, repeat for a different time with a new sample. Analysis of the results was carried out by separating the solids and liquids by precipitation and centrifugation, then the liquid phase obtained was separated by evaporation of solvent until a result with a constant weight (w1) was obtained at 68°C to ensure that the lipid content was free from residual solids, then the results obtained were weighed to calculate the total lipid content and yield.

The solvent used in this research was 40 mL methanol and 90 mL n-hexane; microalgae usually contain lipophilic components such as lipids, pigments, and carotenoids, which are more soluble in non-polar solvents than polar. Therefore, non-polar solvents such as hexane and chloroform are often chosen because of their efficiency in extracting these components [28]. Non-polar solvents, such as hexane and chloroform, are usually more affordable and accessible than polar solvents, such as methanol or acetone, making them a more economical alternative for laboratory use. The fast-dissolving characteristic of non-polar solvents also saves time in the extraction process.

The total lipid content was determined by extracting for 120 minutes with the number of repetitions three times. Entire lipid content can be calculated using equation (1), where the weight of the extracted lipids is the weight of the residue (w1) minus the solids left behind when washing the residue (w2) [29].

$$Lipid\ Content = \frac{(w1 - w2)}{weight\ of\ dried\ microalgae\ (gr)}\ x\ 100\% \quad (1)$$

Meanwhile, the calculation of the yield produced is done by comparing the mass of the extract (gr) with the initial mass of the material before the extraction process (gr) [30]. The yield was calculated according to the Association of Official Agricultural Chemists (AOAC) (1999) in [31] by equation (2) as follows:

$$Yield\ \% = \frac{weight\ of\ extract\ (gr)}{weight\ of\ raw\ materials\ (gr)}\ x\ 100\% \quad (2)$$

3. Results and Discussion

3.1. Total Lipid Content of Microalgae

To determine the total lipid content in an extraction, selecting the proper solvent significantly impacts the efficiency of the lipid extraction process from microalgae. Organic solvents can dissolve lipid content consisting of triglycerides and free fatty acids. Organic solvents can be polar and non-

polar organic solvents. Some types of organic solvents commonly used in the extraction of oil from microalgae include ethanol, acetone, ether, esters, n-hexane, and methanol [27], [32], [33].

The solvent used in this research was a mixture of methanol and n-hexane. Methanol, as a polar solvent, and n-hexane, as a non-polar solvent, were chosen because they have different properties and can complement each other in microalgae extraction. Methanol is effective in extracting polar compounds such as fatty acids and phenolic compounds, while n-hexane is more effective in extracting non-polar compounds such as pigments and carotenoids. In addition, n-hexane has a relatively low boiling point and can dissolve target compounds wholly and quickly [34], [33]. Choosing a mixture of polar and non-polar solvents in microalgae extraction increases the yield and quality of extraction. According to C. L. Teo and A. Idris [35], non-polar organic solvents can degrade microalgae membrane proteins-lipids, while polar organic solvents can degrade protein-lipids. Therefore, mixing polar and non-polar solvents is expected to produce better extraction than using a single solvent [32].

The total lipid content of microalgae was obtained by extracting microalgae for 120 minutes. The results of each stage were obtained by repeating the extraction process three times for each of the same samples. The comparison between the total lipid content and the extracted lipid is shown in Fig.2.

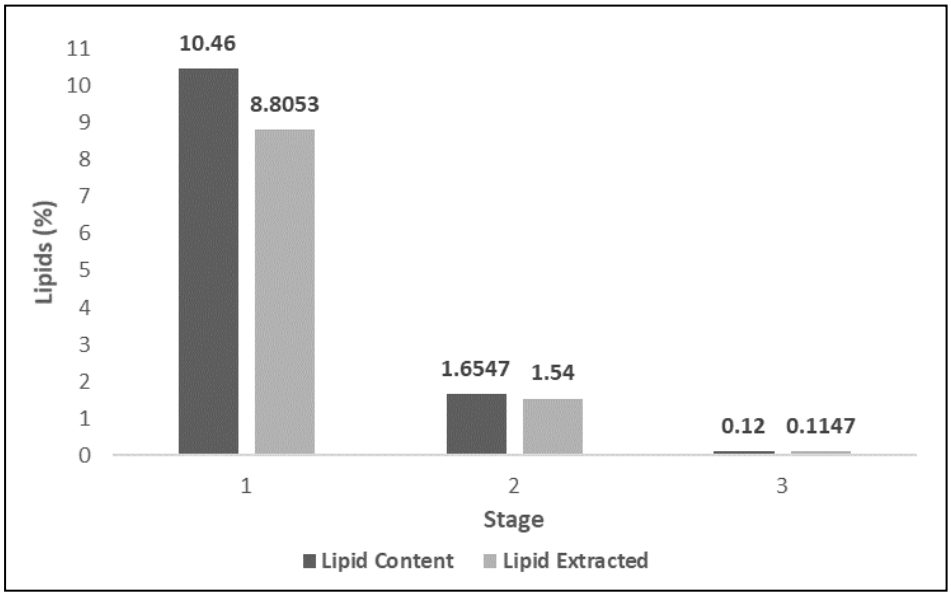

Fig. 2. Comparison of total lipid content and lipid extracted from microalgae *Nannochlopsis sp.*

From Fig. 2. above, it can be seen that a large amount of lipid can be extracted in the first stage, namely 8.8% of the dry weight or 84% of the total lipid present. In the second stage, the remaining lipid is 1.66% of the dry weight and can be extracted from 1.54% or 93% of the total lipids in the second stage feed. Meanwhile, in the third stage, the remaining lipid is 0.12% of the dry weight and can be extracted at 0.1147% or 95% of the existing lipid. These results show that the extraction process with stirring at 2000 rpm for 120 minutes is adequate for extracting lipids. To determine total lipids, extraction using this stirring method carried out in 3 stages can obtain the total lipids present because the remaining lipids are only 0.0053% of the dry weight so that this small amount can be ignored.

3.2. Rate Determination of Extraction Process

The lipid extraction process of microalgae *Nannochloropsis sp.* by stirring method was carried out using a magnetic stirrer at 2000 rpm, with time as the independent variable. The yield of microalgae extraction was obtained using the stirred extraction procedure. The consideration is that the research tools used are straightforward. Fig. 3. shows the effect of extraction time on the percent yield obtained by the stirring method.

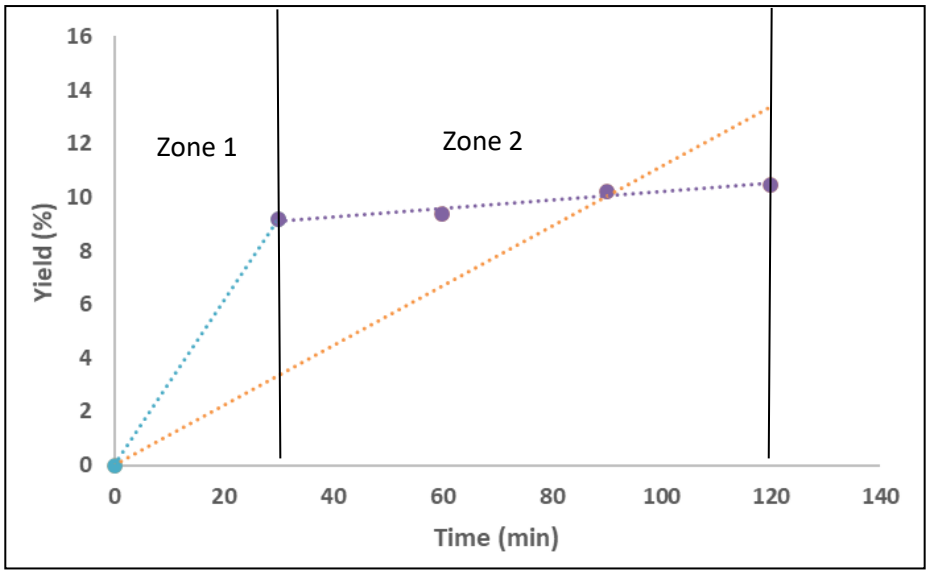

Fig. 3. Effect of extraction time on the percent yield obtained

Fig. 3. shows the effect of extraction time on the percent yield produced. Based on Fig. 3., it is evident that there are two different extraction rates. In the first 30 minutes, a yield of 9.1% of lipid-based on dry weight or 0.6825 grams was obtained, whereas, in the subsequent 90 minutes (30-120 minutes), a yield of 1.3% of lipid-based on dry weight or 0.0975 grams was obtained. Therefore, the extraction rate in zone 1 (0-30 minutes) was 0.023 grams/minute, while in zone 2, it was 0.001 grams/minute.

According to S. Hidayati et al. [36], extraction processes involving cell destruction generally have two extraction zones: the convection zone and the diffusion zone. Zone 1 is from extraction time 0 to 30 minutes. Zone 2 is from an extraction time of 30 to 120 minutes. It can be seen that the optimum rate of extraction is from 0 to 30 minutes, which is about 90% of the lipid gain. This shows that the extraction rate in this section is determined by the release of lipids from the broken microalgae. As for the time from 30 to 120 minutes, the extraction rate tends to slow down or not experience a significant increase. This can mean that the number of broken microalgae in this section decreases.

In addition, the figure shows the percentage yield obtained. The lowest percent yield was acquired at an extraction time of 30 minutes at 9.15%, while the highest percent yield was at an extraction time of 120 minutes at 10.46%. The above results show that the longer the extraction time, the higher the percent yield obtained. The extraction rate above 30 minutes is much smaller than from 0 to 30 minutes, so it is carried out in at most 30 minutes to optimize the extraction process.

4. Conclusion

The microalgae lipid extraction process using the stirring method in this study is to see the extraction effectiveness and the extraction rate characteristics based on the yield produced by microalgae *Nannochloropsis sp.* The extraction process with cell crushing generally has two extraction zones: the convection zone and the diffusion zone. The first zone lasts from the start of extraction to 30 minutes, during which about 90% of the lipids are obtained. In this zone, the extraction rate is determined by releasing lipids from the damaged microalgae. Meanwhile, in the second zone (30-120 minutes), the extraction rate tends to slow as the amount of damaged microalgae decreases. The highest extraction rate occurred in the 30-minute extraction; the rate was reduced after the 30-minute extraction. This shows that the longer the extraction time, the higher the percentage yield obtained. The extraction rate was carried out to optimize the process for 30 minutes. This research highlights the potential of microalgae as a renewable and environmentally friendly energy source. We can make the most of this potential by using the optimum time of stirring extraction methods.

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