An Experimental Study on Microalgae Oil Extraction Through Ultrasound-assisted Algasonic Machine

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Abstract. The rise in fuel consumption has led to a renewable energy crisis, highlighting the importance of finding alternatives to fossil fuels, such as biodiesel. Microalgae, recognized as a promising oil source, plays a pivotal role in third-generation biodiesel. However, efficient, eco-friendly methods are needed to extract oil from microalgae. Among the various techniques, the ultrasound method has shown notable effectiveness in disrupting microalgae cell walls, enabling oil release. Despite its success, its high cost remains a significant challenge. To address this issue, an innovative microalgae oil-extraction machine was developed. The machine incorporates a 40 kHz ultrasound transducer, heater, and automatic control panel. Tests indicated an energy consumption cost of approximately Rp 1,000.00 per process. Using this system, oil yields were achieved at 19.25% oil yield from *Chlorella vulgaris* with a 3.24% Free Fatty Acid (FFA) level, making it suitable for biodiesel. Similarly, *Nannochloropsis oculata* yielded 23.07% oil with a 1.79% FFA level, also suitable for biodiesel. Overall, the *Nannochloropsis oculata* strain demonstrated the highest potential for biodiesel production, with optimal extraction occurring within 180 minutes. This research highlights the potential of ultrasound technology in microalgae oil extraction for biodiesel, offering a cost-effective and a sustainable approach to addressing the energy crisis.

1 Introduction

The escalating energy demands among populations foster a significant decrease in available resources, leading a scarcity of petroleum-based fuels, therefore, drives up fuel prices due to the energy crisis. Studies suggest that with no alternative oil production systems, Indonesia's oil reserves are projected to sustain no longer than 9.5 years [1]. In response to this imminent depletion, biodiesel derived from microalgae emerges as a promising alternative to fossil fuels. Using microalgae as feedstock for biodiesel offers numerous benefits and presents itself as a viable solution to address the impending energy shortages.

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Microalgae represent a class of organisms characterized by a high lipid content, rapid growth rates, and elevated productivity, making them a significant potential for biodiesel production. For optimal result, the study employed *Chlorella vulgaris* and *Nannochloropsis oculata*, two microalgae strains known for the high lipid concentrations. Specifically, *Chlorella vulgaris* exhibits a lipid level of 28% to 32% [2]. Notably, the amount of biofuel produced from microalgae surpasses that of a number of different feedstocks. Microalgae yields up to 58.700 liters of oil per hectare annually, significantly outperforming palm oil, which produces 5.950 liters per hectare per year, and coconut oil, with an output of 2.689 liters per hectare per year [3]. Furthermore, key factors attribute to the oil yield in biodiesel production, including extraction time and temperature, the ratio of solute to solvent, the type of feedstock used, and the frequency of the waves employed during the extraction process.
Ultrasonic waves are frequently employed as an effective method for extracting oil from microalgae. The high pressure generated by ultrasonic waves disrupts the microalgae cell walls, facilitating the release of oil. However, the use of ultrasonic equipment for microalgae extraction is relatively costly, posing a significant barrier to the large-scale production of alternative fuels [4]. Correspondingly, the objective of the study is to develop an innovative technology in the form of a microalgae biomass-to-biodiesel conversion machine called Algasonic. This machine is equipped with an ultrasonic transducer operating at a specific frequency, a heating element, and a control panel that regulate temperature, extraction time, and valve settings within the extraction chamber. The enhanced microalgae extraction technology is designed to identify the optimal strain and extraction time for attaining high yield and superior quality microalgae oil.

2 Method

2.1 Materials

The dried samples of *Chlorella vulgaris* and *Nannochloropsis oculata* were derived from the Brackishwater Aquaculture Fisheries Center (BPBAP) in Situbondo. For the solvent, technical-grade n-hexane (99%), technical-grade methanol (96%), and ethanol (95%) were employed. In addition, a 0.1M solution of technical-grade potassium hydroxide (KOH) was utilized, as well as phenolphthalein (PP) as an indicator and distilled water (aqua demineralized) as the solvent medium. Furthermore, the Algasonic machine was applied as the primary instrument for extraction processes.

2.2 Microalgae oil extraction using an algasonic machine

The Algasonic machine, with dimensions of 38 x 18 x 20 cm (L x W x H), is equipped with an ultrasonic transducer operating at a frequency of 40 kHz, as detailed in Table 1. The microalgal oil was extracted using a solvent mixture of microalgae: methanol: n-hexane (w/v) at a ratio of 1:10:8. This process was performed over three-varied durations of 60, 120, and 180 minutes, with the temperature precisely controlled within 50-60°C. Subsequently, an approximately 60-minute distillation process was employed to separate the pure oil from the solvent. Following this, the extracted microalgal oil was analyzed to identify the free fatty acid (FFA) and oil yield. Each extraction was repeated three times to ensure consistency and reliability of the results.

Table 1. Power Consumption of an Algasonic Machine

No.	Component	Power (W)
1.	Heater Belt	500
2.	Ultrasound Generator	100
3.	DC Motor	10
4.	Omron Timer	11
5.	STC Temperature Sensor	6

To calculate the total electricity consumption in Rupiah, the following formula is applied:

$$Rp = \text{Total energy (kWh) x electricity tariff per kWh} \quad (1)$$

2.3 Analysis and characterization

2.3.1 Free Fatty Acid (FFA) level testing

The Free Fatty Acid (FFA) level in microalgae was measured using a titration method. For biodiesel conversion, the FFA content in microalgae oil is required to remain below 5%. The FFA analysis by titration method involved adding 1 gram of microalgal oil and 25 ml of 95% ethanol into a 250 ml Erlenmeyer flask. Following this, the mixture was heated to a temperature of 40°C. Afterward, titration was conducted using 2 ml of phenolphthalein (PP) indicator and 0.1 N KOH to attain a persistent pink hue, which remained stable for 30 seconds. Additionally, The FFA level was calculated according to the following formula [5]:

$$\text{FFA (\%)} = \frac{ml\ KOH\ x\ N\ KOH\ x\ MW\ x\ 100\%}{sample\ weight\ (gr)\ x\ 1000} \quad (2)$$

Description:
ml KOH: volume of KOH titrant
N KOH: normality of KOH
MW: molecular weight of palmitic acid (256.42 g/mol)

2.3.2 Yield measurement

Yield was calculated through the gravimetric method with the following formula [6].

$$\text{Yield (\%)} = \frac{Oil\ weight\ (ml)}{Sample\ weight\ (ml)} x\ 100\% \quad (3)$$

3 Result and Discussion

3.1 Total electricity consumption

The total electricity consumption was calculated to identify the overall cost required for each component, based on the power consumption and the duration of use. The measured output power for each component is presented in Table 2.

Table 2. Component Power Output

No.	Component	Time (minutes)	Current (A)	Voltage (V)	Power (W)	Energy (Wh)
1.	Heater belt	60	2.27	220	500	500
2.	Ultrasound Generator	60	0.15	12	100	100
3.	Motor DC	60	1.2	12	10	10
4.	Timer Omron	120	0.05	220	11	22
5.	STC temperature sensor	120	0.03	220	6	12
					Total	**644**

Table 2 represents the total energy consumed by each component of the Algasonic extraction machine of 644 Wh, equivalent to 0.644 kWh, with the electricity rate of Rp1,444.70 per kWh. According to the calculations, the electricity cost required for the microalgae oil extraction process is approximately Rp1,000.00 per process.

3.2 Microalgae oil yield using algasonic

The yield of microalgae oil from the extraction process is influenced by a number of factors, including extraction time, the type of solvent, the species of microalgae, and extraction temperature. As displayed in Figure 1, both *Chlorella vulgaris* and *Nannochloropsis oculata* exhibited an increase in yield with longer extraction times. Specifically, *Chlorella vulgaris* yielded 8.18%, 16.12%, and 19.25% at extraction times of 60, 120, and 180 minutes, respectively. Meanwhile, *Nannochloropsis oculata* produced yields of 19.37%, 20.37%, and 23.07% for similar durations.

According to [7], the positive correlation between extraction time and yield is due to the increased collision frequency between atoms and molecules within the system. High-frequency ultrasonic waves propagate through the extraction solvent, thus, changing the pressure. As the pressure drops, gas-filled bubbles form and grow. When these bubbles reach a certain size, the ultrasonic waves can no longer support them, causing the bubbles to burst and release a large amount of energy. This phenomenon leads to the rupture of microalgal cells, allowing lipids to be dissolved by the solvent. Longer extraction duration serves greater opportunity for the solvent to contact and dissolve the extract, thus increasing the oil yield. However, when the yield reaches the maximum threshold, extending the extraction duration has minimal impact or no effect on further improvement [8].

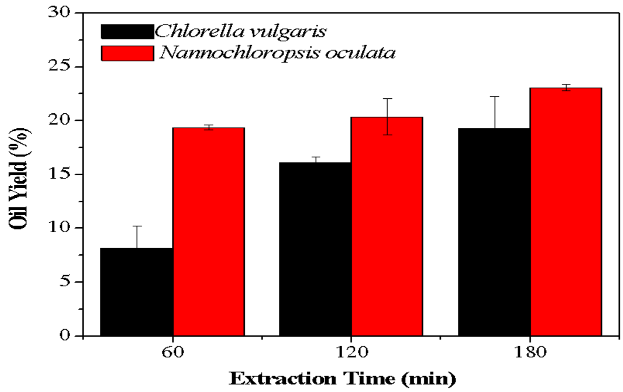

Fig. 1. Oil Yield of Microalgae *Chlorella vulgaris* and *Nannochloropsis oculata*

The microalgae were extracted using a mixture of two solvents: methanol and n-hexane. The polar solvent methanol binds water within the sample, while the non-polar solvent n-hexane dissolves lipids, thereby enhancing the microalgae oil yield [8]. This finding is consistent with the study by [9], suggesting that the optimal conditions for lipid extraction from microalgae involve methanol and n-hexane solvent mixture [9].

In this study, the temperature was maintained at 50-60°C to minimize damage to the oil content. This precaution was necessary as excessively high extraction temperatures can oxidize the microalgae oil, causing decomposition [10]. Furthermore, utilizing excessively high temperatures reduces the oil yield. This is attributed to the boiling points of methanol and n-hexane, which are 64.5°C and 68.7°C, respectively. Thus, temperatures exceeding 60°C may result in solvent evaporation, thereby hindering complete extraction of the microalgae and leading to lower yields [11].

The findings, moreover, indicate that *Nannochloropsis oculata* yields higher oil compared to *Chlorella vulgaris* at 180 minutes of extraction time. This is attributed to the growth rate of *Nannochloropsis oculata* being 1.0 cells/day, whereas *Chlorella vulgaris* has a growth rate of 0.2 cells/day. According to [12], a faster and more optimal growth of microalgae will enhance biomass production, leading to higher oil yields. Additionally, *Nannochloropsis oculata* exhibits greater biomass productivity, at 1.8 g/L/day, compared to *Chlorella vulgaris*, which has a productivity of 0.3 g/L/day. The higher biomass production signifies larger raw material is available for extraction, thereby enhancing the yield [13].

3.3 Free Fatty Acid (FFA) test

The lipid level of the microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* dissolved in methanol and n-hexane, was analyzed to identify the free fatty acid (FFA) level using the titration method. The free fatty acid concentration was established based on the amount of KOH required to induce a color change in the sample. As illustrated in Figure 2, the FFA level in the *Chlorella vulgaris* microalgae oil at extraction durations of 60, 120, and 180 minutes was 9.36%, 4.50%, and 3.24%, respectively. In contrast, the FFA level in the oil extracted from *Nannochloropsis oculata* over similar durations was 2.47%, 2.13%, and 1.79%, respectively. The permissible FFA standard for microalgal oil intended for transesterification into biodiesel is <5%, ensuring a low saponification number [14].

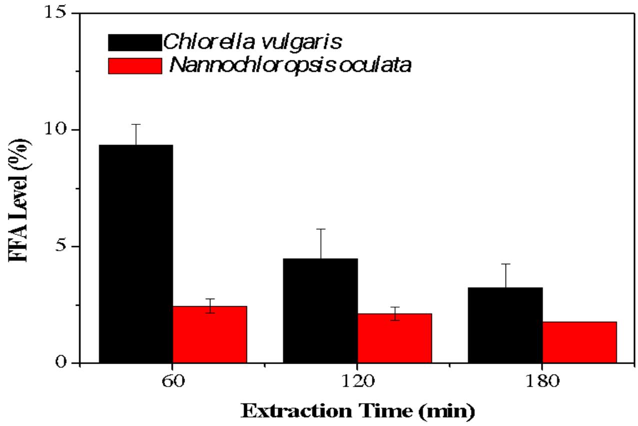

Fig. 2. The Graph of the Relationship Between Extraction Time and FFA level in Microalgal Oil from *Chlorella vulgaris* and *Nannochloropsis oculata*

The results suggest that the microalgal strains that adhere the specified criteria were observed on *Chlorella vulgaris* at extraction durations of 120 and 180 minutes, and *Nannochloropsis oculata* at extraction durations of 60, 120, and 180 minutes. The optimal FFA level was obtained from *Nannochloropsis oculata* after 180 minutes of extraction. This finding aligns with a study that indicates longer extraction process generally leads to higher FFA removal efficiency. However, prolonged extraction durations potentially increase the FFA levels, signifying that an optimal extraction length is required to achieve FFA concentration that adhere biodiesel standard specifications [15]. The FFA level is closely linked to the interaction between the solvent and the microalgal lipids, with extended extraction durations facilitating greater FFA removal from the extraction medium [16].

FFAs are primarily formed through the hydrolysis of triglycerides by water, either due to high extraction temperatures or enzyme catalysis. The presence of FFA serves as an early indicator of oil degradation attributed to hydrolytic reactions and subsequently stimulate oxidative deterioration [17,18]. A higher FFA concentration is associated with lower biodiesel quality. Biodiesel exhibits lower stability due to the less resistance to oxidation, in addition to being corrosive, thus, posing higher risk of engine damage. Moreover, elevated FFA levels potentially lead to saponification during the transesterification process when reacting with a base catalyst, thereby reducing the biodiesel yield [19].

In this study, the high FFA levels observed in *Chlorella vulgaris* microalgae extracted at 60 minutes is presumably attributed to environmental factors during the algae's growth, such as temperature, light, pH, aeration, salinity, and nutrient availability. For FFA levels exceeding 5%, esterification is required prior to biodiesel conversion [14]. Additionally, a comparison of ultrasonic extraction technology with other extraction technologies is presented in Table 3.

Table 3. Comparison of Preceding Technologies

Algae Species	Method	Yield (%)	FFA (%)	Reference
Chlorella vulgaris	Supercritical Fluid Extraction (22-26°C, 9 hours)	1.81	3.85	[20]
	Ultrasonication (3 hours)	11.52	-	[21]

	Microwaves (100°C, 5 mins)	18	-	[22]
	Soxhlet Extraction	18	-	[23]
	Algasonic (50°C, 3 hours)	**19.25**	**3.24**	**This study**
Nannochloropsis oculata	Soxhlet (40°C, 18 hours)	9	-	[24]
	In Situ Transesterification (70°C, 2 hours)	4.21	0.52	[25]
	Osmotic Extraction (60°C, 5 hours)	4.14	-	[26]
	Microwave (80°C, 20 mins)	17.80	9.60	[27]
	Algasonic (50°C, 3 hours)	23.06	**1.79**	**This study**

4 Conclusion

The Algasonic machine designed for this study demonstrated its effectiveness in achieving a microalgae oil yield of 19.25% for *Chlorella vulgaris* with a Free Fatty Acid (FFA) level of 3.24% within three hours. In contrast, *Nannochloropsis oculata* produced a higher yield of 23.07% with a lower FFA level of 1.79% over the same duration. The high yield and low FFA level of *Nannochloropsis oculata* highlight its superior potential for biodiesel conversion. Furthermore, the use of the Algasonic machine is regarded as both efficient and cost-effective, as evidenced by the total electricity consumption cost of Rp 1,000.00 per extraction process. To enable its application at an industrial scale, it is necessary to upscale the machine to a continuous-type operation. Additionally, further testing is required to assess the composition of dried microalgae and its potential impact on the final oil yield.

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