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Mass Transfer Phenomena during the Ultrasound-assisted Extraction of Algal Oil from *Spirulina sp.*

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Abstract. Recently, microalgae are the potential to be developed because it is easy to cultivate. This study investigated the effect of the solvent ratio on the mass transfer of algal oil at various residence times from 15 minutes to 60 minutes during the ultrasound-assisted extraction process at 25 kHz and stirred at 150 rpm. The highest amount of the algal oil yielded was 3.01%, obtained by a ratio of isopropyl alcohol to n-hexane of 2:3 ratio at 60 minutes. A mathematical model was applied to obtain the mass transfer coefficient, diffusivity coefficient, and Henry's constant contributing to the mass transfer rate. A GC-MS analysis was conducted to show the type of fatty acids and the fatty acid methyl ester produced by the extraction that showed the trans-esterification reaction. The lowest yield was obtained by 3:1 isopropyl alcohol to n-hexane ratio, which had the lowest k_ca and D_e (4.80×10^{-5} min^{-1} and 2.00×10^{-9} cm^2/min, respectively).

Keywords: Algal oil; Fatty acid; Mass transfer; Spirulina sp; Ultrasound-assisted extraction

1. Introduction

Microalgae, containing lipids, has become one of the potential renewable energy resources (Setyawan *et al.*, 2018; Chisti, 2008). They are potentially developing in Indonesia, which has an extensive water area. Besides, they can grow in fresh and saltwater (Daneshvar *et al.*, 2018; Clemens *et al.*, 2011). The proteins, lipids, and carbohydrates contained in a microalga reach 6-52%, 7-23%, and 7-23%, respectively, depending on the strain type and growth condition (Dewati *et al.*, 2022; Kang *et al.*, 2011). Microalgae are classified into *Cyanophycae*, *Bacillariophyceae*, *Chrysophycae*, and *Chlorophyceae* based on their pigments. They also have high photosynthetic efficiency, rapid growth rate, and small cultivation area (Clarens *et al.*, 2010). *Spirulina sp.* is one of the green algae found in various environments, like brackish water, freshwater, and seawater, and thus easy to cultivate in Indonesia (Rilisa and Suantika, 2021)

Microalgae are the third generation of biomass resources (Sardi *et al.*, 2022), the first and second of which have disadvantages. The first comes from food, such as palm oil, corn oil, canola oil, and bean, and creates a conflict regarding the fulfillment of food and energy (Haque *et al.*, 2015; Mohr and Raman, 2013). Meanwhile, the second comes from non-food materials such as jatropha and waste biomass containing cellulose, which require a high

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operational cost to produce energy (Jamilatun *et al.,* 2020; Balan, 2014).

Lipids in microalgae comprise the storage (non-polar) lipids and the structural (polar) ones that include oils (Sharma, Schuhmann, and Schenk, 2012). The storage ones have the primary form of triglycerides as the energy source (D'Alessandro and Antoniosi-Filho, 2016). A polar lipid is the membrane's primary structure that plays a role in metabolism (Casal and Oliveira, 2007). Non-polar lipids, such as free fatty acids (saturated, unsaturated, and polyunsaturated) and acylglycerol (mono-acyl, di-acyl, and tri-acyl), can be easily esterified (Asikainen *et al*., 2015). In contrast, polar lipids, such as glycolipid and phospholipid, have long carbon chains and are highly unsaturated, thus uneasily converted into biodiesel using conventional methods (Atadashi *et al.*, 2012).

The ultrasound-assisted extraction method often functions to get algal oils from microalgae cells by cavitation (Vilkhu *et al.*, 2008). It is environmentally friendly since it takes less time and saves more energy than conventional extraction methods (Sela, Budhijanto, and Budiman, 2021; Adam *et al.*, 2012). Ultrasound energy produces chemical and physical effects because cavitation bubbles collapse. These effects can lead to biodiesel production (Suganya, Kasirajan, and Renganathan, 2014).

However, an organic solvent is required for lipid extraction (Pradana *et al.*, 2020; Wang *et al.,* 2014) by first examining its polarity, surface tension, viscosity, and vapor pressure (Lavilla and Bendico, 2017). Extracting polar and non-polar lipids needs a mixture of polar and non-polar solvents like chloroform and methanol as the most frequently used solvents for lipid extraction from any living tissue for their high speed and nature is quantitative (Halim, Danquah, and Webley, 2012).

The oil extracted can be processed as biodiesel as a renewable energy resource. The cetane number of biodiesel is higher than that of petroleum diesel (Guo *et al*., 2016). Besides, the combustion emission profile in biodiesel is better, meaning that it is good for the environment. These reasons make biodiesel an excellent alternative to fossil fuels (da-Silva *et al.*, 2012).

Research on the ultrasound-assisted extraction of lipids from microalgae found that the sonication amplitude and duration of lipid extraction affected the percentage of lipid extraction from *Dunaliella sp*., which had a positive correlation (Shahi *et al.*, 2021). The analysis of the fatty acid profile of algal oil through an ultrasound-assisted Soxhlet extraction from microalgae showed the potency for producing biodiesel (Wong and Shahirah, 2019). The power, time, and pulse were the most dominant factors in the yielded oil developed by STATISTICA software (Wiyarno, Mohd-Yunus, and Mel, 2014). However, the mass transfer phenomena during ultrasound-assisted extraction have not been studied, especially in algal oil extraction. This research aimed to observe the effect of the ratio of the polar solvent to the non-polar one on the yield and the mass transfer phenomena.

2. Materials and Methods

2.1. Materials

The microalga used in this study was *Spirulina sp.* obtained from Nogotirto Algae Park Sleman, Yogyakarta, Indonesia. The components of this biomass were identified by the proximate analysis, as shown in Table 1. Before the extraction, the microalga was sifted to 0.06 mm to enlarge the contact area so that the cell walls would be broken during the extraction process by ultrasonic waves (Liu *et al*., 2022; Lavilla and Bendicho, 2017).

The solvents used were *n*-hexane technical grade, Merck, and isopropyl alcohol 99.5%, Merck. The technical grade n-hexane solvent was used because it cost less than the pro-analytic grade and is enough to extract the lipid. While isopropyl alcohol 99.5% was used because the trans-esterification reaction was expected to happen.

2.2. Methods

This extraction method used an ultrasound device that contained a generator, transducer, stirrer, and ultrasonic probe. The ultrasonic probe and generator generated the ultrasonic waves at 100 Watt. Cavitation bubbles were released using a transducer, producing high shear stress to break cells (solid phase). The sample was placed in an ultrasound device for 15, 30, 45, and 60 minutes at a frequency of 25 kHz and a stirring speed of 150 rpm. The solvents used were mixed isopropyl alcohol and *n*-hexane with ratios of 1:3, 2:3, 1:1, 3:2, and 3:1.

After the extraction process, the separation process was needed to separate the solid and liquid phases through vacuum filtration. After the filtration, the oil and solvent had to be separated. The separation process used a distillation apparatus at 80^{0}C. The oil condensed while the solvent evaporated. The algal oil was weighed until it got a constant weight. After the weighing, the oil sample was analyzed using GC-MS to identify the type of fatty acid. The scheme of the extraction process is illustrated in Figure 1.

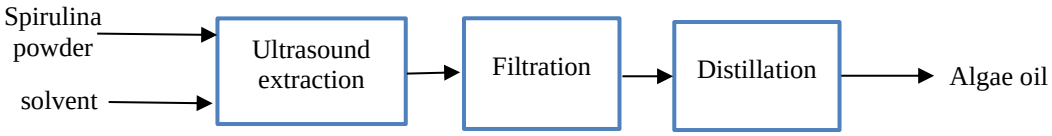

Figure 1 Extraction Process

The oil concentration from the extraction process was calculated using Equation (1).

$$C_f = \frac{m}{V} \quad (1)$$

Here, C_f was the concentration of extract in a liquid phase (g/mL), m was the mass of extract (g), and V was the volume of liquid (mL).

3. Results and Discussion

3.1. Oil Yield

In this study, oil yield refers to the essential indicator to evaluate extraction efficiency. The oil extracted from microalgae was processed into biodiesel as a renewable energy resource. There are two groups of solvents: polar and non-polar (Saini *et al.*, 2021). The former included methanol, ethanol, and isopropyl alcohol, while the latter included chloroform and n-hexane. The solvents used in this study were chosen based on polarity (high polarity was polar solvent, and the low polarity was non-polar solvent), boiling point temperature to reduce energy consumption during the separation process, and toxicity to ensure the solvent was safe (González-Fernández *et al.*, 2020). Their properties are shown in Table 1. Although all the solvents had suitable polarity index and boiling point temperature, which should be below the boiling point of algal oil (about 180 ^{0}C when using the boiling point of petroleum diesel), safety must be under consideration.

LC_{50} referred to toxicity. The lower number of LC_{50}, the more toxic the substance. LC_{50} of isopropyl alcohol is extremely higher than methanol because methanol is toxic and can form high vapor concentrations at room temperature, which can be easily absorbed through respiration during exposure (Moon, 2017). It is essential to choose a safer solvent to minimize the hazard in the process and environment. Moreover, methanol causes high environmental pollution in the transesterification process based on a life cycle assessment study (Wahyono *et al.*, 2022). So, isopropyl alcohol was chosen as the polar solvent (polarity index was 3.92) and n-hexane as the non-polar solvent (polarity index was 0.1), although LC_{50} does not correlate with mass transfer phenomena.

The oil yield was calculated by Equation (2). The mass of lipids, protein, and carbohydrates in microalgae was calculated using the proximate analysis data shown in Table 2.

Table 1 Properties of Solvent

Solvent	Polarity Index	Boiling point	LC_{50} (inhalation, rat)
methanol	5.1	64.7 ^{0}C	128.2 ppm, 4 hours
isopropyl alcohol	3.92	82 ^{0}C	>10,000 ppm, 6 hours
chloroform	2.7	61 ^{0}C	125 ppm, 4 hours
n-hexane	0.1	68.7 ^{0}C	48,000 ppm, 4 hours

Table 2 The results of proximate analysis of Spirulina powder

Component	Content (%)
Water	11.25
Ash	6.63
Lipid	0.81
Protein	48.32
Carbohydrate	32.99

$$yield = \frac{mass\ of\ extract}{mass\ of\ lipid,protein,and\ carbohydrate\ in\ microalgae} \times 100\% \quad (2)$$

The extraction using a single solvent was examined in this experiment, shown in Figure 2. Isopropyl alcohol with a polarity index of 3.92 was used as a polar solvent, while *n*-hexane with a polarity index of 0.1 was taken as the non-polar one. The yield of the polar lipid solvent was higher than that of the non-polar one because the polar lipids contained in the microalga were more than 50 percent of the total lipids (Arif *et al.*, 2019; Breuer *et al.*, 2013). Besides, other polar cellular components, such as protein, pigments, and carbohydrates (Irawati *et al.*, 2020; Agustini *et al.*, 2015), were also present in this extraction product. The yield at 60 minutes of extraction time reached 1.31% using isopropyl alcohol and 0.72% using *n*-hexane. The yield of n-hexane solvent was not saturated at the long extraction time, indicating the mass transfer rate using n-hexane was relatively slow.

The effect of the mixed solvent on the oil yielded is illustrated in Figure 3, indicating that the ratio of the polar solvent to the non-polar one affects the yield. The lowest yield was at the ratio of 3:1, showing that the extraction process needs a non-polar solvent more than a polar solvent. This finding was in contrast to the extraction yield using a single solvent. The non-polar solvent, if being too much, can lower the yield. The ratio of the solvents, therefore, should be optimized. Based on the experiment, the highest yield was 3.01% when the extraction ran under the ratio of 2:3.

In an extraction using mixed solvents (in this case, isopropyl alcohol and n-hexane), both are added simultaneously to the microalgae biomass to extract both polar and non-polar lipids. Besides, the aqueous phase contains non-lipid components, such as proteins and carbohydrates (Wang *et al.*, 2021; Halim, Danquah, and Webley, 2012).

The comparison using a single solvent and a mixed solvent is shown in Table 3. The highest yield from a single solvent was achieved by using isopropyl alcohol at 60 minutes of extraction (1.31%), while the lowest yield using mixed solvent at the same extraction time was 1.46%. It was indicated that using mixed solvent gave a higher yield.

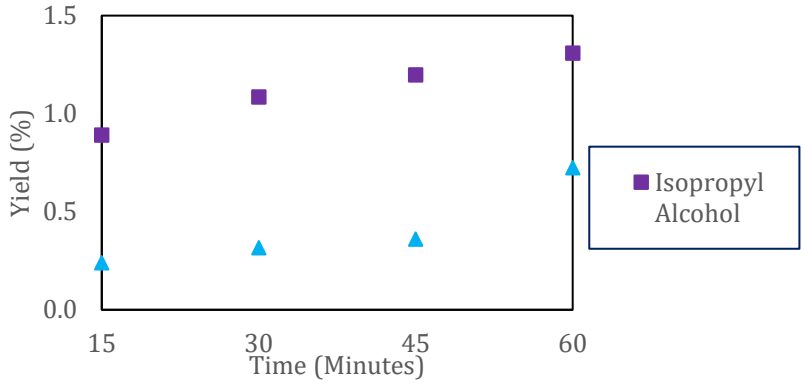

Figure 2 Algal Oil Yield by Single Solvent Extraction

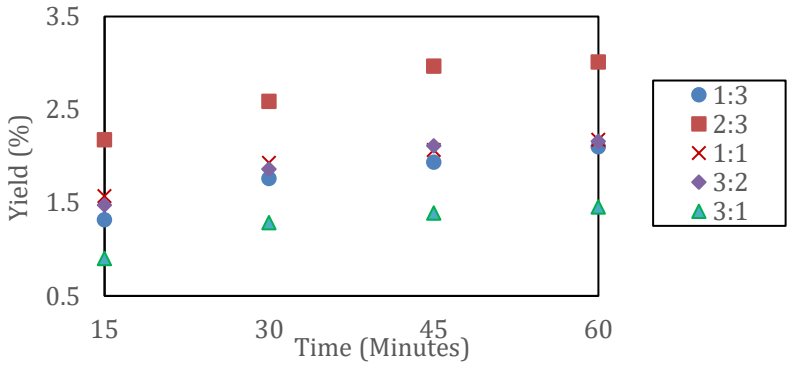

Figure 3 Algal Oil Yield by Solvent Mixture Extraction

Table 3 The Comparison of Yield Using Single Solvent and Mixed Solvent

Yield at 60 minutes of extraction	Single solvent	Mixed solvent
Maximum yield	1.31	3.01
Minimum yield	0.72	1.46

The ratio of microalgae mass to solvent volume at the 3:2 polar to non-polar solvent ratio is illustrated in Figure 4. Three solid-to-solvent ratios were investigated namely 1:1, 2:3, and 3:10 biomass-to-solvent ratios. The highest yield was 2.18%, achieved by the 3:10 biomass-to-solvent ratio, while the lowest yield was obtained by an extraction process using the 1:1 biomass-to-solvent ratio.

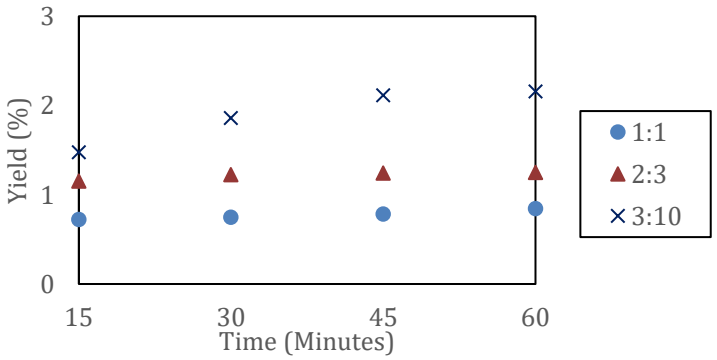

Figure 4 Algal Oil Yield in Various Biomass to Solvent Ratios

3.2. Product Composition

Algal oils produced by various isopropyl alcohol to *n*-hexane ratios were analyzed based on the % area of the peak in the GC-MS analysis results, as shown in Figure 5. The

algal oils had three groups of components: fatty acids, hydrocarbons, and alcohol. The classification was based on the name of the compound and its molecular formula.

Overall, the hydrocarbon composition was relatively constant except in the 3:1 isopropyl alcohol to *n*-hexane ratio. It declined from above 50% to 42.10%. Meanwhile, the highest alcohol peak area was found in the extracted oil with the 3:1 isopropyl alcohol to *n*-hexane ratio.

The highest %peak area of fatty acid and its derivative products was found at the 3:1 isopropyl alcohol to *n*-hexane ratio, while the lowest was at the 1:3 ratio. These findings indicated that *Spirulina sp.* has polar lipids more than non-polar lipids because polar lipids pull the polar substances.

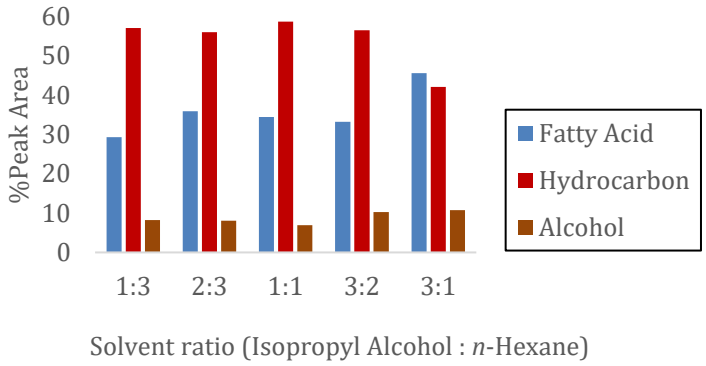

Figure 5 The Composition of Algae Oil

The fatty acid compositions are shown in Table 4. In this study, the compositions of fatty acid (FA) and fatty acid methyl ester (FAME) in algal oil were palmitic acid, linoleic acid, methyl palmitate, and methyl linoleate.

Table 4 The Compositions of FA and FAME in Algae Oil

Solvent (isopropyl alcohol to *n*-hexane) ratio	FA		FAME	
	Palmitic Acid	Linoleic Acid	Methyl Palmitate	Methyl Linoleate
1:3	11.99	8.61	2.78	1.17
2:3	8.17	19.53	3.58	4.65
1:1	7.72	18.79	2.03	5.86
3:2	6.17	19.15	2.78	5.12
3:1	5.81	26.87	6.30	5.69

The FAME yielded from the fatty acid, as illustrated in Figure 6, reached the highest amount when using the 3:1 isopropyl alcohol to *n*-hexane ratio, with the highest amount of alcohol solvent. This phenomenon indicated that the trans-esterification reaction that reacted isopropyl alcohol and fatty acid (FA) into fatty acid methyl ester (FAME) occurred in this extraction process.

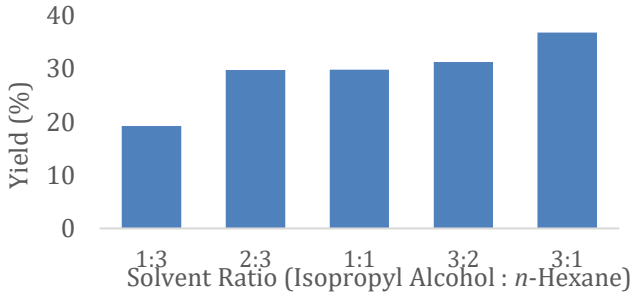

Figure 6 FAME Yield to FA in Algal Oil

3.3. Mass Transfer Modeling

Algal oil extraction is a mass transfer phenomenon from the solid (microalga cells) to the liquid (solvent). Such a phenomenon can be modeled by Equation 3, and the equilibrium equation can be approached by Henry's law as in Equation 4 as follows:

$$\frac{dm}{dt} = k_c a(C_f^* - C_f)V \tag{3}$$

$$C_A = HC_f^* \tag{4}$$

In the solid phase, the mass transfer can be arranged from Equation 5 to Equation 8 with the assumption that microalga's shape was round and the operation condition was isothermal.

$$\left(-D_e 4\pi r^2 \frac{\partial C_A}{\partial r}\Big|_r\right) - \left(-D_e 4\pi r^2 \frac{\partial C_A}{\partial r}\Big|_{r+\Delta r}\right) = 4\pi \overline{r}^2 \Delta r \frac{\partial C_A}{\partial t} \tag{5}$$

$$\lim_{\Delta r \to 0} \frac{r^2 \frac{\partial C_A}{\partial r}\Big|_{r+\Delta r} - r^2 \frac{\partial C_A}{\partial r}\Big|_r}{\Delta r} = \frac{\overline{r}^2}{D_e} \frac{\partial C_A}{\partial t} \tag{6}$$

$$\frac{\partial}{\partial \mathrm{r}}\left(\mathrm{r}^2 \frac{\partial \mathrm{C_A}}{\partial \mathrm{r}}\right) = \frac{\mathrm{r}^2}{\mathrm{D_e}} \frac{\partial \mathrm{C_A}}{\partial \mathrm{t}} \tag{7}$$

$$\frac{\partial^2 C_A}{\partial r^2} + \frac{2}{r}\frac{\partial C_A}{\partial r} = \frac{1}{D_e}\frac{\partial C_A}{\partial t} \tag{8}$$

Equations 3, 4, and 8 are simultaneous differential equations that have boundary conditions, as in Equations 9, 10, and 11.

$$\text{At t = 0, } C_A(r, 0) = C_{A0} \tag{9}$$

$$\text{At t > 0, } -D_e \frac{\partial C_A}{\partial r}(R, t) = k_c a(C_f^* - C_f) \tag{10}$$

$$\frac{\partial C_A}{\partial r}(0, t) = 0 \tag{11}$$

Here, $k_c a$ is volumetric mass transfer coefficient (min^{-1}), C_f is the concentration of lipid in a liquid phase (g/ml), C_f^* is the saturated concentration of lipid in a liquid phase (g/ml), C_A is lipid concentration in the solid phase (g/ml), H is Henry constant, r is the radius of microalgae particles (cm), D_e is effective diffusivity (cm^2/min).

MATLAB processed these simultaneous differential equations to get $k_c a$, D_e, and H constants, as shown in Table 5.

Table 5 Constants of $k_c a$, D_e, and H in various solvent ratios

Solvent (isopropyl alcohol to *n*-hexane) ratio	$k_c a$ (min^{-1})	D_e (cm^2/min)	H
1:3	1.31×10^{-3}	1.11×10^{-7}	8.57
2:3	1.48×10^{-4}	9.39×10^{-7}	12.61
1:1	1.64×10^{-4}	5.61×10^{-6}	8.83
3:2	1.52×10^{-4}	6.10×10^{-7}	8.87
3:1	4.80×10^{-5}	2.00×10^{-9}	10.04

The highest mass transfer coefficient, Henry's constant, and effective diffusivity were reached when the algal oil was extracted at the 1:3, 2:3, and 1:1 isopropyl alcohol to *n*-hexane ratios, respectively. All the data obtained concluded that D_e and H, besides $k_c a$, are factors in the oil yield. The lowest oil yield was obtained by 3:1 isopropyl alcohol to n-hexane solvent ratio, which had the lowest number of $k_c a$ (4.80×10^{-5} min^{-1}) and D_e (2.00×10^{-9}), while Henry's constant was the second highest (10.04). At this solvent ratio, the GC-MS'

peak area of the fatty acid result was the highest. It indicates that other solvent ratios extracted more other compounds than lipids.

4. Conclusions

The yield of mixed solvent was higher than single solvent. When using a single solvent, polar solvent achieved a higher yield than non-polar solvent. The ratio of solvents in algal oil extraction affects the oil and substances yielded. In this study, the highest oil yield, namely 3.01%, was obtained at the 2:3 isopropyl alcohol to n-hexane ratio. The products yielded were not only affected by the mass transfer coefficient but also by the effective diffusivity coefficient and Henry's constant. Based on the extraction process yields analysis, the large amount of the polar solvent indicated that polar lipids dominated the fatty acid in the algal oil and were converted to biodiesel under a trans-esterification reaction through the ultrasound-assisted extraction process. This statement was confirmed by the high amount of fatty acid methyl ester yielded using the highest volume of alcohol.

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