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Extraction of Algae Oil from *Nannocloropsis* sp.: A Study of Soxhlet and Ultrasonic-Assisted Extractions

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Abstract: This study aims at describing the characteristics of the microalgae oil extraction from Nannochloropsis sp. using soxhlet and ultrasonic. There were two extraction processes that were investigated, Soxhlet-assisted Extraction (SE) and Ultrasonic-assisted Extraction (UE) and both used ethanol solvent. A combination of several criteria was selected to find the characteristics of each extraction. In the SE, a variety of ethanol concentration and time were used; whereas in the UE, a variety of ethanol volume, time and temperature were applied. The given frequency for all UE treatments was 40 kHz. The quality of algae oil proceeded by SE was shown by the level of FFA (Free Fatty Acid) and saponification number. In the SE study, the best combination was gained when the ethanol concentration was 70% and the given time was 200 min in which the FFA level was 9.4% and the saponification number was 286.8. While in the UE study, 51.6 min, 98% of ethanol concentration and 69.62°C were the best circumstance in which the quantity of the oil yield got its maximum. In SE, the higher solvent concentration, the higher FFA level and saponification number were gained. However, after reaching the peak at particular circumstance, the saponification number decreased gradually. Meanwhile, UE reduced the length of extraction time.

Key words: Microalgae oil, soxhlet extraction (SE), ultrasonic assisted extraction (UAE), free fatty acid (FFA), saponification number

INTRODUCTION

Microalgae are microscopic algae that have one cell and chlorophyll and live in colony. They commonly live in fresh water and sea water (Richmond, 2004). They varied in sizes, starting from a few micrometers (μm) to a few hundreds of micrometers. They do not have roots, stems and leaves; however they use photosynthesis process to turn the sun light, carbon dioxide and water into lipid, carbohydrate, protein and oxygen. Some microalgae species are able to produce products like antioxidants, sterols, enzymes, polymers, peptides, toxins and fatty acids. Fatty acid, exists in lipid, is one major component for biodiesel production.

The existence of lipid in microalgae is prominent. Some of microalgae are potential sources of biodiesel fuel due to their high lipid level (Li *et al*., 2008; Chisti, 2007; Sheehan *et al*., 1998). One of them is marine microalgae *Nannochloropsis* sp. which lipid level is 54% (Feinberg, 1984) and 12-53% (Mata *et al*., 2009). Moreover, their lipid productivity level is 27.00 mg/L/day (Brennan and Ownde, 2009) and 37.6-90.0 mg/L/day (Mata *et al*., 2009). Marine algae contain a bewildering array of major fatty acids. The major saturated fatty acid is invariably palmitate (C16:0) and, in contrast to higher plants, palmitoelate (C16:1) is the major monoene. C18 fatty acids are much less abundant than in leaves and the C20 polyunsaturated acids are very important (Gunstone *et al*., 2007). Fatty acid components of *Nannochloropsis* sp. which was used in this study, from the highest to the lowest are palmitoleat acid (C16:1) 20-43.82%, palmitate acid (C16:0) 9-23.89% and oleic acid (C18:1) 4-12.25% (Pratono, 2008; Gunstone *et al*., 2007).

Extraction is commonly used to obtain oil from plants. Some of the well known extraction methods are pressure, sokhlet, osmosis pressure, microwave, supercritical and ultrasonication (Shah *et al*., 2005; Szentmihalyi, 2001). Indeed, each extraction method has

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its strengths and weaknesses. Among them, soxhlet and ultrasonic methods are used in this study. Soxhlet is simple but less economical. Meanwhile, ultrasonic extraction is less time consuming as it has a relatively shorter operational time (Shah *et al*., 2005) comparing to the conventional extraction (Dong *et al*., 2004). Moreover, ultrasonic needs a low operational temperature. A high temperature results more oil but its quality is low (Liauw *et al*., 2008).

Solvent was used in both methods; it has a great influence to the result (Wiyarno *et al*., 2009; Jadhav *et al*., 2009). Ethanol (Chaiklahan *et al*., 2008) is normally used to extract microalgae using ultrasonic energy.

MATERIALS AND METHODS

Material: The main material used in this study is the powder of *Nannochloropsis* sp. (which was taken from *Balai Besar Laut (BBL)* Lampung, Indonesia). Initially, the powder was washed to remove the coagulant that was usually used during the harvest. Next, the powder was dried by putting them for 8 h at 80°C. Besides the microalgae powder, ethanol was used as solvent in the soxhlet assisted extraction process (BP Grade; 79°C, BM; 46.07 g moL^{-1}).

Sohklet Extraction (SE): In SE study, a variety of ethanol concentration and time were used. The solvent concentration varied, ranging from 66 to 94%. Meanwhile, the time variations of the soxhlet circulation were 80, 100, 150, 200 and 220 min. The oil quality proceeded by SE would be shown by the level of FFA (Free Fatty Acid) and saponification number. The extraction process includes several important steps. First, microalagae powder was placed in a thimble filter paper; then it was put into the 250 mL soxhlet Next, ethanol was added; the ratio was 1:3 g mL. Finally, distillation was applied to separate oil from solvent.

Ultrasonic Extraction (UE): In this UE study, the equipment used was ultrasonic cleaning bath. It was the JAC ultrasonic type 1505 with 150 W/200W of nominal power, 300×150×150 mm of bath dimension, 40 kHz of frequency and 5.7 L of volume. While the given frequency for all of the UE treatments was 40 kHz, a variety of ethanol volume, time and temperature were applied. The ratio of powder to solvent was 1:3, 1:5 and 1:10 g mL. The time span used for sonication was 10, 20 and 30 min. The oil extraction process was conducted at three different temperatures i.e. 23, 40 and 60°C.

In the UE process, the microalgae powder which was put in a closed glass was placed in the ultrasonic cleaning bath. Then the UE procedures were applied. The extracted liquid was separated manually and the filtrate was evaporated using rotary vacuum evaporator at 60°C.

Design and analysis: The experiment was design by Box-Behnken design of industrial statistics and Six Sigma of STATISTICA 6.1 to get the optimum value of each extraction model. Analysis on the extracted oil was done by measuring the content of free fatty acid (%FFA) and Saponification Number (SN). Gas Chromatography Mass Spectrometry (GCMS) was used to analyze the FA composition.

RESULTS AND DISCUSSION

Soxhlet extraction

Effect of different circulation (time) and ethanol concentration on quantitative yield: The quantitative result was measured from the volume of extracted oil per powder weight (v/m). Figure 1 shows the relationship between amount of circulation (time) and concentration of ethanol with the oil yield (% of dried algae). The equation bellow shows the correlation between algae oil yield (Y, %) with the effect of circulation and ethanol concentration after response surface methodology process.

$$Y = -81.1151+4.4377*A+0.0544*A^2+1.9919*B-0.0084*B^2- 0.0720*A*B$$

where, Y is algae oil (%), A is circulation and B is ethanol concentration.

It is clear from the Figure 1 that the more time circulation the more oil was extracted (run No. 3). This is in contrast with the effect of ethanol concentration to the oil (run No. 8). The more ethanol concentration, the least oil was yielded. This finding is in line with the result of some studies concerning ethanol solvent (Abdullah *et al*., 2010) that there will be an increase in extraction result as the ethanol concentration increase but in a certain high ethanol concentration, the amount of oil extracted will decrease. The finest oil concentration in this study was gained when the ethanol level was 70%, at that time the extracted oil was 8-13 mL (16-26% of algae weight). When the ethanol concentrations were 90 and 94% the resulted oil was 5 mL algae oil (10% of algae weight). It happened because the selectivity of the ethanol would firstly increases; then after a certain circumstance it would decrease. The thick concentration made other components such as phospholipids and chlorophyll were taken; it can be seen from the color of the extract which turned greener, thicker and more turbid. The high

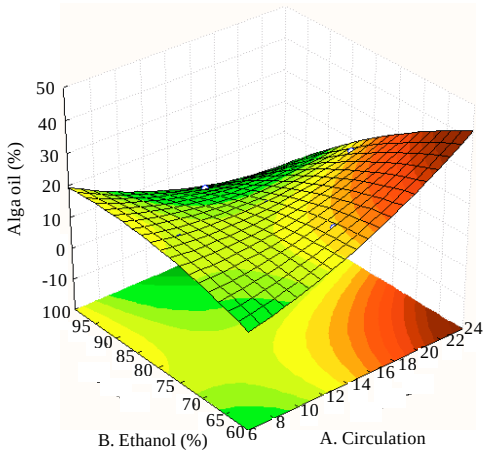

Fig. 1: Relationship between amount of circulation (time) and concentration of ethanol with the oil yield

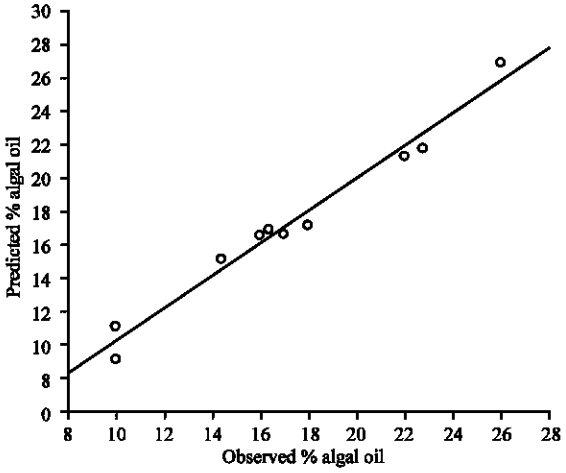

Fig. 2: A Comparative plot between experimental and predicted surface area

temperature also caused the oil turbidity; while the increase of circulation time immediately increased the resulted oil. The more contact time of the material, the more oil would be extracted.

Figure 2 shows the relationship between experimental values versus predicted values using the model question developed (Choong, 2009). A line of unit slope, the line of perfect fit with points corresponding to zero error between experimental and predicted values is also shown in Fig. 2. The coefficient of correlation (R^2) is 0.9770. The result in Fig. 2 show that the regression model equation provides an accurate description of experimental data, indicating that it has successfully captured the correlation between the two parameters (time and concentration) to the surface area.

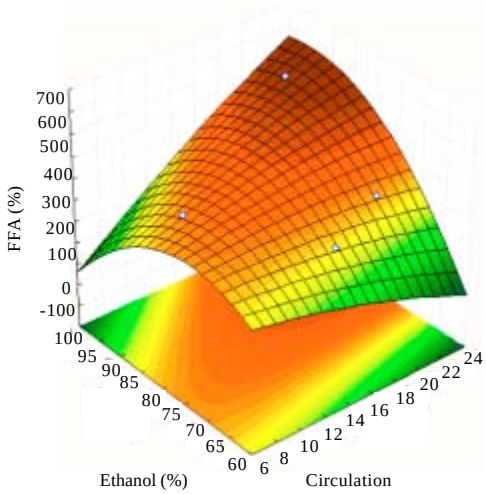

Fig. 3: Circulation (time) vs. % solvent vs. % FFA

Effect of time and solvent concentration on qualitative yield: The Free Fatty Acid (FFA) and saponification number of the algae oil was analyzed to identify its quality. Saponification number is the amount of alkali needed for the saponification of some sample oil (Ketaren, 2008). For biodiesel basic material, the lower the FFA, the better oil quality is. The high level of FFA will disturb the process of biodiesel production. While the high saponification number indicates good oil quality to be used as basic material of biodiesel.

Figure 3 shows the correlation among the amount of circulation, solvent concentration and the FFA level gained. The long duration of circulation time made the oil contact the heat which then influenced the oil quality. Thermal oxidation affects the oil quality. Peroxide accumulation in the algae oil at 100-115°C temperature is two times bigger than at 10°C temperature; furthermore it also makes the increase of FFA and level of carbonyl oxygen in the oil (Ketaren, 2008).

Figure 3 also shows that there is a relationship between ethanol concentration and fatty acid level. The higher ethanol concentration, the higher fatty acid level will be.

Figure 4 shows the relationship between the ethanol concentration and the saponification number. From that Fig. 4 it is clear that, to some extent, more circulation time or contact time led to a better saponification number. It also happened to ethanol concentration as its increase also makes the saponification number increase, however, at particular concentration the number will decrease.

Ultrasonic assisted extraction

Effect of temperature and solvent volume: The effect of ethanol solvent and temperature to the microalgae extraction process was shown in Fig. 5. Similar to SE,

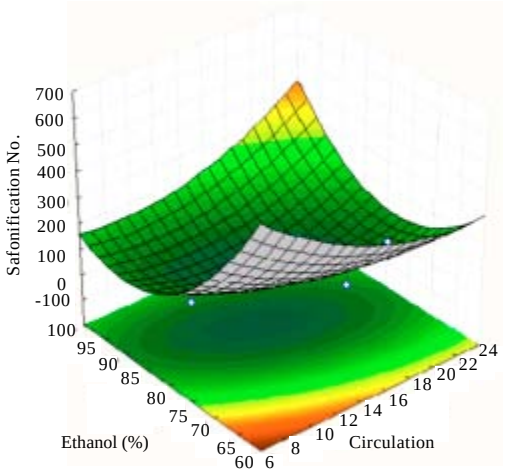

Fig. 4: Circulation time vs. % solvent vs. saponification number

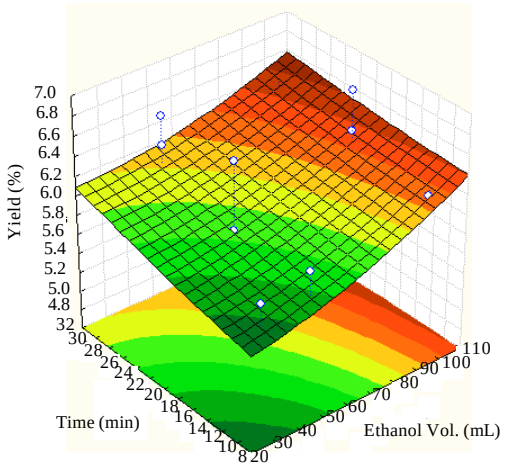

Fig. 6: Effect of ethanol volume and time on the oil yield

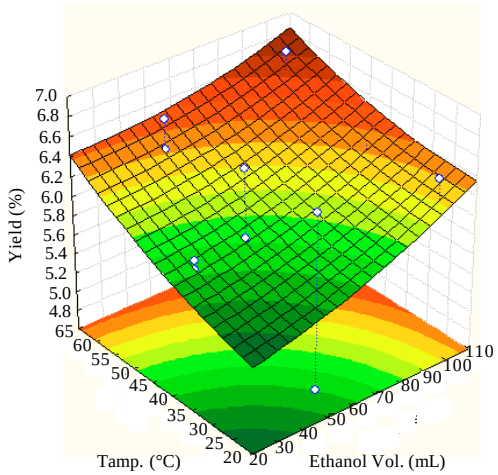

Fig. 5: Effect of temperature and ethanol volume on the oil yield

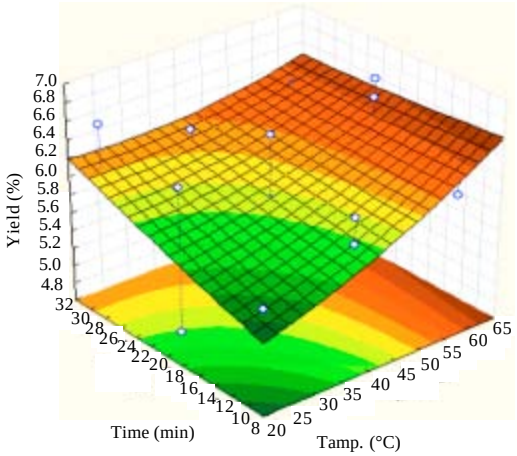

Fig. 7: Effect of temperature and time on the oil yield

ethanol solvent also contributes to the enhancement of the yielded oil. The more solvent volume, the more the extracted oil will be. The increase of temperature level causes the oil yield drastically as shown in the Fig. 5. Temperature contributes the rapidity of oil released from the microalgae cells as they dissolved. However, as shown in the Fig. 5, 96% of ethanol which is categorized as high level, was not a good solvent composition for SE. It is because ethanol at this percentage has bigger polarity but has lower level of selectivity, so the ethanol not only takes the oil but also takes other components of the microalgae cells, such as phospholipids and chlorophyll.

Effect of time and ethanol volume on the oil yield: Figure 6 shows the effect of time to the oil yield by ethanol solvent. Given the same volume (50 mL) and same temperature (60°C), the resulted oil proceed within 20 min was 0.06 gram than 10 min, despite the decrease at min 30. The addition of time will increase the amount of oil but not very significant (Suslick, 1989). This was indicated by the rise of the oil with less drastic time (Fig. 6).

Effect of temperature and time on oil yield: Figure 7 shows the effect of temperature and extraction time to the oil yield. This figure shows that the effect of temperature and time were significant to ethanol solvent. From Table 1, it is clear that with ethanol solvent, temperature-

Table 1: Effect estimate of parameters using ethanol solvent

Factor	Effect	S.E	T (5)	p-value	-95% Cnf.Limt	+95% Cnf.Limt	Coeff	S.E Coeff	-95% Cnf.Limt	+95% Cnf.Limt
Mean/Interc	6.155625	0.216681	28.40876	0.000001	5.59863	6.712620	6.155625	0.216681	5.59863	6.712620
(1)Ethanol (L)	0.501503	0.639859	0.78377	0.468658	-1.14331	2.146314	0.250751	0.319930	-0.57165	1.073157
Ethanol (Q)	-0.053537	0.489603	-0.10935	0.917179	-1.31210	1.205028	-0.026769	0.244802	-0.65605	0.602514
(2)Temp (L)	0.565093	0.470467	1.20113	0.283489	-0.64428	1.774466	0.282546	0.235233	-0.32214	0.887233
Temp (Q)	-0.075344	0.384319	-0.19605	0.52292	-1.06327	0.912578	-0.037672	0.192159	-0.53163	0.456289
(3)Time (L)	0.266211	0.487488	0.54609	0.608478	-0.98692	1.519338	0.133106	0.243744	-0.49346	0.759669
Time (Q)	-0.018128	0.370657	-0.04891	0.962886	-0.97093	0.934676	-0.009064	0.185328	-0.48547	0.467338
1L by 2L	-0.138701	0.755072	-0.18369	0.861472	-2.07968	1.802273	-0.069351	0.377536	-1.03984	0.901136
1L by 3L	-0.096717	0.632634	-0.15288	0.884471	-1.72295	1.52952	-0.048358	0.316317	-0.86148	0.764760
2L by 3L	-0.322246	0.659961	-0.48828	0.646014	-2.01873	1.374242	-0.161121	0.329981	-1.00936	0.687121

Table 2: Critical values of variables using ethanol

Factor	Observed minimum	Critical values	Observed maximum
Ethanol	30	66.99770	100
Temp (°C)	23	69.62172	60
Time (min)	10	51.60132	30

Table 3: Oil content from *Nannochloropsis* sp.

Fatty acid	Name	Molecule mass (g moL^{-1}) (MMfa)	% in sample	Molecule mass contribution (g moL^{-1}) (Mmc)
C 12	Dedecanoic acid	200.30	16.300	32.649
C14	Tetradecanoic acid	288.40	11.143	25.451
C16:0	Hexadecanic acid	256.42	12.517	32.069
C18:0	Octadecanoic acid	284.40	5.626	16.000
C18:1	Octadecenoic acid	282.46	43.492	112.847
C18:2	Octadecadinoic acid	280.45	5.858	16.428
Average molecular mass of constituent fatty acid (Mmfa)				235.444

time combination was significant to generate the oil comparing with the other combinations. This can be seen from p value which is relatively small i.e. 0.0646014. This data shows that the high level ethanol solvent (96%) is not good to be used as the solvent in algae oil extraction (Liauw *et al*., 2008). The optimum time and temperature which is shown in Table 2, are at 51.60 min and 69.62°C, respectively. So, comparing to the sokhlet extraction, ultrasonic extraction has shorter time and needs lower temperature.

***Nannochloropsis* sp. fatty acid composition:** Similar microalgae strain might have different component composition and level. This is affected by different cultivation technique and environment. *Nannochloropsis* sp. used in this study contains 0.46% of carbohydrate, 11.18% of water, 60.02% of ash, 4.33% of protein and 4.77% w/w of lipid. GCMS test toward the oil component resulted from this microalgae can be seen in Table 3.

Molecular mass of Microalgae oil (MMoil) was determined using the formula:

$$MMoil = [3MMfa+MMgly]-3MM$$

$$Water = [3(235.444)+92.1]-3\ (18) = 745\ gram\ moL^{-1}$$

CONCLUSION

The study of sokhlet-assisted extraction revealed that the ethanol concentration influenced the algae oil quality (as indicated by FFA level and saponification number). The better result was gained when the ethanol concentration was 70%. Meanwhile, the amount of circulation also influences the quality of oil yield. The amount of optimum time was 200 min or 3.3 h. Ethanol selectivity increased gradually as its concentration raised. To some extent, the increase of concentration decreased the selectivity. While the use of ultrasonic in extraction reduced the time and temperature significantly; it needed only 51.6 min in 69.62°C. The GCMS test of algae oil component indicated that there was no significant difference between both methods of extraction. Further study toward the oil quality resulted from both methods need to be conducted.

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