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A Study on The Ultrasonic Oil Extraction and Insitu Transesterification of Microalgae Biodiesel

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Abstract

The extraction and transesterification of microalgae oil are interesting topics-besides culturing and microalgae strain- in the development process of biodiesel microalgae. This is an experimental laboratory study that was run using ultrasonic homogenizer Omni Ruptor 4000, examining the effect of type of solvent, solvent concentration, alga-solvent ratio, ultrasonic power, ultrasonic time, ultrasonic pulse and mixing toward yield. Based on Box-Behnken design, a quadratic model is developed to correlate the parameter to surface area to analyze certain factors and combination of dominant factors.

The result shows that power, time and pulse as the most dominant factors that influence the yield. In the extraction, the combinations of pulse-time give better result than power-pulse combination. While in the in situ transesterification, the power-time combination give better result that power-pulse combination. Even though the optimum point has not been reached yet, in general the combination of power-time is categorized as the most influential combination to increase the yield.

The experimental values versus predicted values use the model equation developed by STATISTICA Software version 6.0. A line of unit slope, the line of perfect fit with points corresponding to zero error between experimental and predicted values is also shown that the coefficient of correlation (R^2) is 0.97977 (for extraction) and 0.98743 (for in situ). The density of Nannochloropsis sp is 0.924 g/ml, saponication number is 114, 269 KOH/1 g oil. The percentage of FFA is 19.67% consisting of monounsaturated and polyunsaturated Octadecenoic acid (C18:1) 43.49%, Dedecanoic acid (C12) 16.30%, Hexadecanoic acid (C16:0) 12.51%, Tetradecanoic acid (C14) 11.43%, Octadecadinoic acid (C18:2) 5.85% dan Octadecanoic acid (C18:0) 5.62%.

Key words: *Interaction factor, Ultrasonic Assisted, Oil Alge Component and Characteristic*

Introduction

The need of fuel keeps increasing as world population growth; however it is not balanced with the production of world's oil and liquid gas. It is predicted that in 2020 the production of both types of fossil fuels is declining [1]. These conditions encourage countries in the world to perform efficiency and explore the potential of new energy source and perform diversification of fuel oil. Biofuels, like biodiesel, play a very important role as alternative energy and green energy. It can replace petroleum fuel nowadays. It can be produced from organic material, such as plants, and can be renewed. High quality biofuel, in general, can be obtained from the feedstock, not the food stock. This can reduce 50% emission of greenhouse effect comparing to fossil fuel. Microalgae, as one source for biofuel, it is an example of potential renewable energy source.

Algae are plants which have one or more cells; they have chlorophyll and live in colony. They use photosynthesis process to turn the sun light, carbon dioxide as well as some nutrition from water into lipid, carbohydrate and protein and release the oxygen. Nannochloropsis sp is one type of microalgae which is potential for basic material of biofuel [2] [3]. Some studies report that their lipid level is 54 % [2], and 12-53% [3]. The lipid productivity level of nannocloropsis sp is 37.6-90.0 $mg.L^{-1}.day^{-1}$ [3].

Extraction is one method used to obtain/squeeze oil from plant. Some of the wellknown extraction models are pressure, solvent/sokhlet, osmosis pressure, microwave, supercritical and ultrasonication [4]. Transesterification is the conversion of glycerol ester (triglycerides) to monoester of lower alcohols, typically methanol or ethanol. Another alternative to the conventional process, which is considered to have potential of

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reducing the processing units and costs of the fuel conversion process, is the '*in situ*' Transesterification method. The *in situ* process facilitates the conversion of the biomass oil to FAME directly from the oil bearing biomass.

It is known that high temperature will comprise more but low quality oil [5] and conventional extraction takes a longer time [6]. Microalgae flour of *nannochloropsis sp* is used in this study. Cavities resulted by ultrasonic sound trigger crash or collision among particles in the body of the cell, which furthermore increase the heat, then this heat breaks the cell, and finally releases the oil from the cell.

Material and Method

Material

The type of algae used in this study is *Nannochloropsis sp*. Its powder was previously dried in room temperature. Ethanol used as solvent in in this research (BP Grade; 79^{o}C, BM; 46.07 g/mol).

Ultrasonic Extraction (UE) and Ultrasonic In-situ Transesterification (UiT)

The mixture of 25 grams of microalgae and 150 ml of ethanol solvent. That was run using ultrasonic homogenizer Omni Ruptor 4000, examining the effect of type of solvent, solvent concentration, alga-solvent ratio, ultrasonic power, ultrasonic time, ultrasonic pulse and mixing toward yield. The extracted liquid was separated manually. Then, filtrate was evaporated using rotary vacuum evaporator at 60 ^{0}C then analyzed using GCMS.

ANOVA

ANOVA is used to uncover the main and interaction effects of categorical independent variables (called "factors") on an interval dependent variable. A "main effect" is the direct effect of an independent variable on the dependent variable. An "interaction effect" is the joint effect of two or more independent variables on the dependent variable. Whereas regression models cannot handle interaction unless explicit cross product interaction terms are added, ANOVA uncovers interaction effects on a built-in basis

Analysis of algae oil and Algae fuel

Analysis on the extracted oil was done by measuring the content of FFA component on algae oil and algae fuel

Result and Discussion

1. Screening of Factors

1.1 Most Influential Factor

Using Box Behnken Design of Statistica Software, the screening was done toward the finding most influential factors in extraction and *in-situ* transesterification process. This process was to get the dominant factor among many factors in this research. Based on the data analysis, the result is as shown in the Pareto chart (Figure 1).

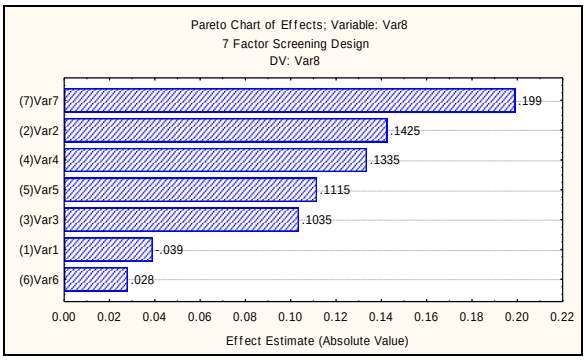

Figure 1. Figure Pareto Chart of Effect

Table 3.1, it can be understood that the most influential effects are (1) Variable 7 (Ultrasonic Pulse), (2) Variable 2 (Ultrasonic Time), (3) Variable 4 (Ultrasonic Power), (4) Variable 5 (Type of Solvent), (5)

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Variable 3 (Solvent Purity), (6)Variable 1 (Ratio), and (7) Variable 6 (Mixing), respectively. Furthermore, three of the most influential factors were selected. These are (1). Ultrasonic pulse, (2). Ultrasonic power and (3). Ultrasonic time.

2. Interaction of Ultrasonic Variable on Oil Yield

2.1 Effect of Power and Pulse on the Oil Yield

The effect of ultrasonic power dan ultrasonic pulse on the oil yield resulted in the extraction process can be seen in Figure 2a and the *in situ* process can be seen in Figure 2b. In the extraction process (Figure 2a), on the effect of ultrasonic power, it is clear that the increase of ultrasonic power drastically increase the amount of oil yield. This result was also shown by some other researchers [7] [8]. Meanwhile, in the ultrasonic pulse, the increasing of ultrasonic pulse increases the yield, but at some points the increasing of the pulse will decrease the percentage of yield. The optimum result was gained when the pulse was 75% and yield of 0.1%.

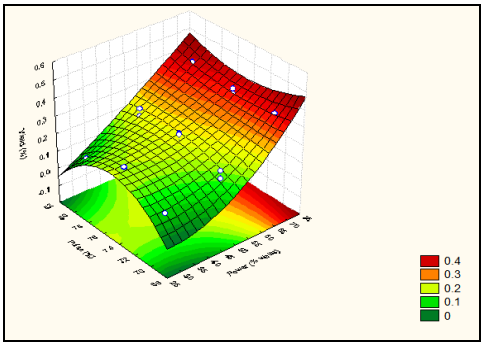

Figure 2a. Effect of ultrasonic power and pulse on oil yield (extraction process)

In the *in situ* process (Figure 2b), it is seen that the increasing of ultrasonic power and pulse will increase the yield gained. Both variables tend to approach their optimum points. The increase of ultrasonic power results in significant yield comparing to ultrasonic pulse.

In the graph of ultrasonic power influence on oil yield, there is a significant increase in the early treatment and it tends to be stagnant (the stable graph) when the power is at 70% capacity (210 W). It also happens to the graph of ultrasonic pulse to the yield, which tends to be stable when the power is at 75% capacity. The result indicates that the conversion of yield increases linearly with the rise of ultrasonic power and ultrasonic pulse and at a faster rate with power than pulse.

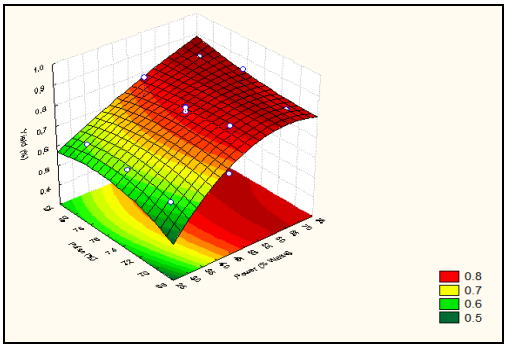

Figure 2b. Effect of ultrasonic power and pulse on FAME yield (*in situ* process)

2.2 Effect of Power and Time on the Yield

Similar case also happens in the oil extraction from flaxseed in the similar frequency (20 KHz) and similar solvent (methanol) as there was not significant increase [7]. The significant increase of oil yield occured in the first 30 minutes. The same thing was also shown by hepiridinextracion in Penggan (Citrus reticulata) peel. The different result was only shown by different power [8]. This is because the strength of the power of ultrasonic is more able to break the microalgae cell wall and yield the oil than the contact duration between cell and the solvent. Therefore in Figure 3 it is indicated that the increasing of ultrasonic power is more significant than ultrasonic time.

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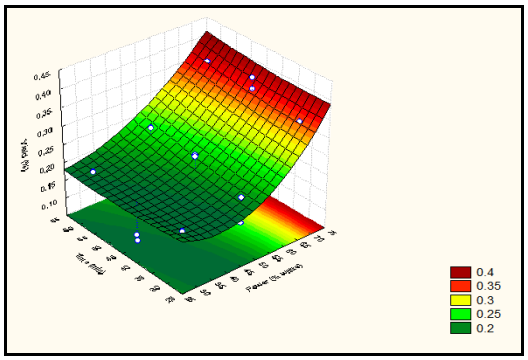

Figure 3. Effect of ultrasonic power and time on oil yield (extraction process)

Different result was shown in the *in situ* process (Figure 4) in which the increase of both variables did not give a significant effect on the yield, but the correlation between the two tends to show optimizing graph of the *in situ* process that had been conducted.

In the effect of duration of extraction time, the graph tended to be optimum, in which the increase of time will increase the oil yield in the beginning of the process, but then it tended to decrease at particular point. The yield tends to be increasing at minute 30 up to 60 and decreasing at minute 60 up to 85. This is similar to the previous researches which reported that the yield reached the optimum after 40 minutes [10]. Meanwhile, Widjaja, *et al* [11] has reported that the optimum point was reached at 15x3 minutes and it tends to decrease after 30x3 minutes.

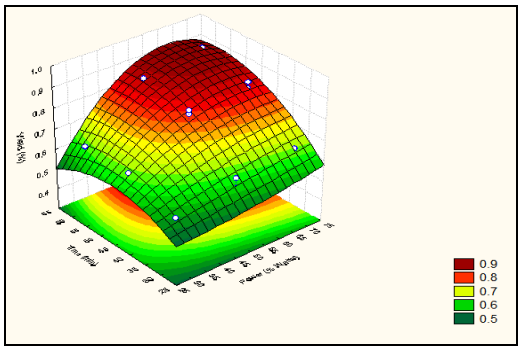

Figure 4. Effect of ultrasonic power and time on FAME yield (*in situ* process)

2.3 Effect of Variable Time and Pulse on Yield

In the extraction process (Figure 5) it is seen that the increasing of pulse results in the increasing of the yield up to particular limit (75% pulse). After this, the increasing of pulse tends to decrease the oil yield. It is different with the result shown by variable time, in which the increasing of time will give the increasing of yield.

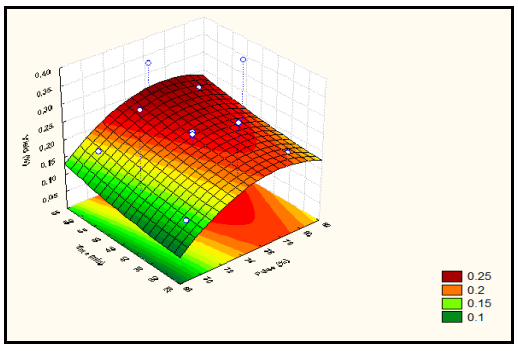

Figure 5. Effect of ultrasonic time and pulse on oil yield (extraction process)

In the *in situ* process (Figure 6), it is clear that the influence of ultrasonic time is more significant than pulse. In this case, it is also clear from the graph that pulse addition tends to reduce the yield. In

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combination with pulse, it was shown that the more the time used, the more yield will be gained. This condition was slightly different as time length was combined with Ultrasonic power. This shows that the correlation between time and power gives more significant result than correlation between time and pulse.

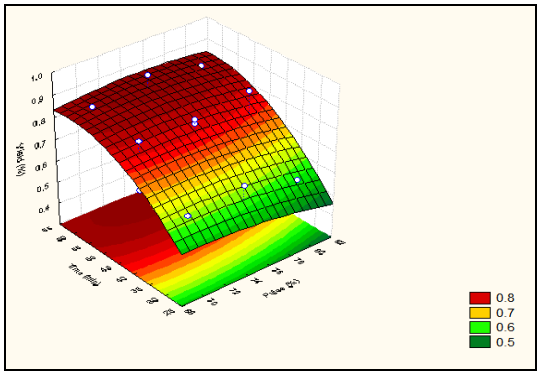

Figure 6. Effect of ultrasonic time and pulse on FAME yield (*in situ* process)

From all interactions in the extraction process and *in situ* transesterification (Figure 4) there is similarity in the effect of each variable. The increase of ultrasonic power and ultrasonic time tends to increase the yield as well. Both variables are more dominant in the extraction process and *in situ* ultrasonic transesterification toward the yield resulted. Figure 7 shows the ultrasonic power in the optimization of microalgae oil extraction is the most dominant variable comparing to ultrasonic time and ultrasonic pulse, as *p* value was 9.0286. While in Figure 8 it is shown that ultrasonic power and ultrasonic time as the most dominant variable comparing to ultrasonic pulse as *p* values were 4.126066 and 4.057866.

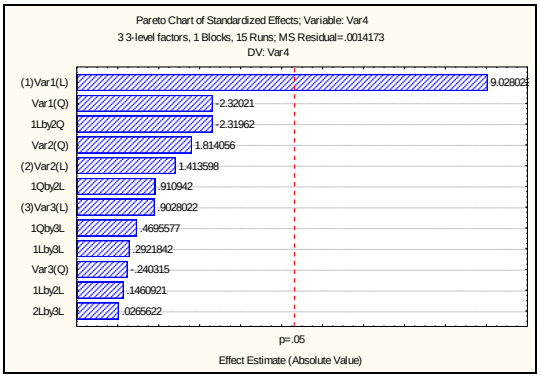

Figure 7. Pareto Chart of Effect on Extraction process

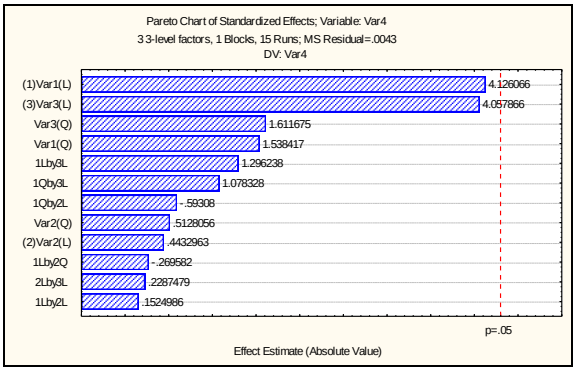

Figure 8. Pareto Chart of Effect on *In situ* Transesterification Process

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3. ANOVA for Response

From the ANOVA for response surface quadratic model for surface area, the model *p*-values of 0.002344 (extraction) and 0.000215 (*insitu*) imply that the models are significant. Figure 3 and 4 shows the experimental values versus predicted values using the model equation developed by STATISTICA Software version 6.0. 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 Figure 9. The coefficient of correlation (R^2) is 0.97977 (extraction) and 0.98743 (*in situ*). The results in Figure 9 and 10 demonstrate that the regression model equation provides an accurate description of the experimental data, indicating that it has successfully captured the correlation between the three parameters to oil and biodiesel yield.

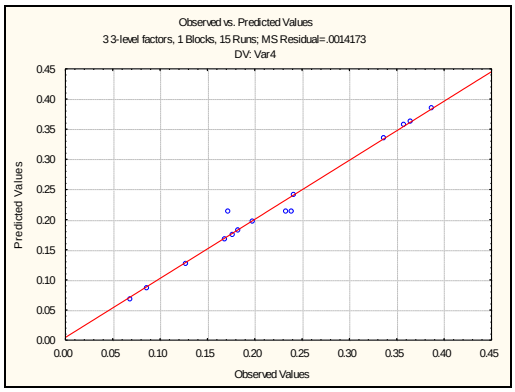

Figure 9. A comparative plot between experimental and predicted oil yield for extraction

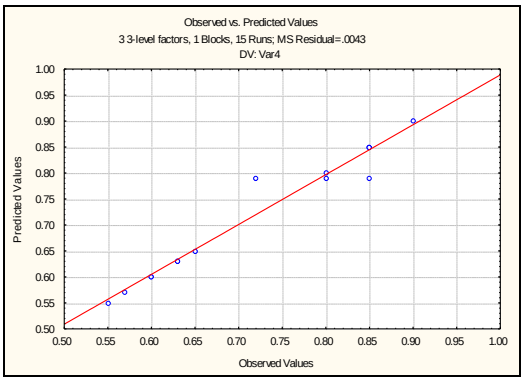

Figure 10. A comparative plot between experimental and predicted oil yield for *in situ*

4. Chemical Component of Microalgae Oil

The major fatty acid compositions of the tested microalgae were determined using GCMS Analysis (Tabel 4.6). These results are consistent with the reported by Yon, *et al*, (2009) In the three tested microalgae, oleic acid (C18:1) and linoleic acid (C18:2) were commonly dominant. The FA content of microalgae depends on the strains and the culturing condition.

To estimate the mean molecular mass of the constituent lipid fatty acids (MMFA). With the formation of the triglyceride molecule facilitated by the combination of fatty acid molecules and a molecule of glycerol with the condensation of three molecules of water, the average molecular mass of the microalgae oil (MM oil) can be calculated using Eq. 1

$$MMoil = [3MMfa+MMgly]-[MMwater] \quad (1)$$

where, $MM_{glycerol}$ and MM_{water} represent the molecular masses of glycerol and water, respectively. The average molecular weight of the *Nannochloropsis sp* lipid was calculated to be 745 gram/mol.

Conclusion

This research was done to investigate the effect of ultrasonic use in the extraction process and *in situ* transesterification process of microalgae into biodiesel. This research consists of two steps, screening and

optimizing. Seven factors were used in the screening stage namely microalgae-solvent ratio, ultrasonic time, solvent concentration, ultrasonic power, type of solvent, mixing and ultrasonic pulse. In the optimizing process, three significant factors from screening process were taken to determine the optimum condition. The Box Bhenken Design of STATISTICA version 6.0 was used in both stages to analyze the result.

The main conclusions that can be drawn from this study are summarized as follow:

1) In the screening process, the most influential factors in the ultrasonic assisted factor and *in situ* transesterification factor respectively from the most to the least are ultrasonic pulse, ultrasonic time, ultrasonic power, type of solvent, solvent concentration, microalgae-solvent ratio and mixing.
2) The variable of ultrasonic power in the optimization process (ultrasonic extraction assisted) of microalgae oil extractions is the most significant variable comparing to the ultrasonic time and ultrasonic pulse (*p* value = 9.0286). Meanwhile in the *in situ* ultrasonic power and ultrasonic time are more significant than ultrasonic pulse (*p* value = 4.126066 and 4.057866, respectively).
3) From all interactions in the extraction process and *in situ* transesterificationthere is a similarity in the effect of each variable. The increase of ultrasonic power and ultrasonic time tends to increase the yield as well. Both variables are more significant in the extraction process and *in situ* ultrasonic transesterification toward the yield resulted.
4) The chemical composition of fatty acids from*Nannochloropisis sp* strain consists of monounsaturated and polyunsaturated Octadecenoic acid (C18:1) 43.49%, Dedecanoic acid (C12) 16.30%, Hexadecanic acid (C16:0) 12.51%, Tetradecanoic acid (C14) 11.43%, Octadecadinoic acid (C18:2) 5.85% and Octadecanoic acid (C18:0) 5.62%.
5) Oil algae characteristic have density of 0.924 g/ml, Saponification Number 114, 269 KOH/1 g oil and 19.67% FFA.

Acknowledgement

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Tabel 1. Fatty acid composition of N*annochloropsis* sp

No.	Fatty Acid	Name	Molecule Mass (g/mol) (MMfa)	% in Sample	Molecule mass Contribution (g/mol) (MMc)
1.	C12	Dedecanoic acid	200.30	16.300	32.649
2.	C14	Tetradecanoic acid	288.40	11.143	25.451
3.	C16:0	Hexadecanic acid	256.42	12.517	32.069
4.	C18:0	Octadecanoic acid	284.40	5.626	16.000
5.	C18:1	Octadecenoic acid	282.46	43.492	112.847
6.	C18:2	Octadecadinoic acid	280.45	5.858	16.428
Average molecular mass of constituent fatty acid (MMFA)					235.444

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