Optimization of biodiesel production from *Chlorella sp* through in-situ microwave-assisted acid-catalyzed trans-esterification

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Abstract. Microalgae is one of the potential raw materials in producing the third generation biodiesel thanks to its high lipid contents and the fact that it requires relatively small space for cultivation. Microalgae in the form of *Chlorella sp*. was used as a raw material in this study due to its high oil content, i.e. up to 30% of the dry algae weight. The use of microwave irradiation in this process would accelerate the in-situ trans-esterification reaction by extracting microalgae lipids and simultaneously converting them into Methyl-Esters. This study aims to investigate the methyl ester production through the in-situ trans-esterification process by studying the effect of acid catalyst concentration (0.2 – 0.5 mol/L), microwave power (300 – 600 W) and reaction time (30 – 90 minutes). The experiment was carried out in a 500 ml flat bottom flask made of pyrex, under the influence of microwave irradiation in which homogeneous sulfuric acid (H_2SO_4) was introduced as a catalyst. The experiment was carried out in atmospheric pressure with the following operating variables: catalyst concentration, microwave power and reaction time, respectively. Prior to running the experiment, the response surface methodology using Box Behnken Design (3 factors and 2 levels) was conducted beforehand in order to minimize the number of runs. It is suggested from the analysis that the optimum conditions for the in-situ microwave-assisted trans-esterification of Chlorella sp. with sulfuric acid catalyst are as follows: microwave power (370 W), concentration of catalyst (0.2 mol/L), and trans-esterification time (82.7 min) with yield of 63.36 %. The predicted yield values generated from the response surface methodology with Box-Behnken design exhibit a high degree of confirmation with the actual yield from the experiment, suggesting that the optimization methodology carried out has made the experiment more effective and efficient by focusing only on certain specific parameters in order to get the best results, in terms of both quality and quantity.

1. Introduction

The raw materials of biodiesel have developed into several generations over time. The first generation of biodiesel uses materials derived from palm oil, corn oil, olive oil, etc., i.e. materials that are normally used as food. The raw materials of the second generation of biodiesel come from plant oils that are not edible due to the presence of components that are hazardous to health or toxic (non-food), such as jatropha oil, nyamplung oil (callopylum inopylum), thus, they would not compete with food availability. However, they require a large area for cultivation and a long time to harvest. The third generation biodiesel is derived from oil extracted from microalgae, which does not require extensive area for cultivation and have a relatively short harvesting time. Harvesting of microalgae can be done every day.

Microalgae are the fastest organisms in photosynthesis and contain high lipid content. They can produce up to 200 times more results than other plants [1]. Microalgae have recently become promising candidates as a third generation biodiesel feedstock, as an alternative raw material for producing large amounts of biomass energy [2]. Various types of microalgae have been cultivated in Indonesia, especially in areas around East Java, including Dunaliella, Chlorella sp. and nanochloropsis [3].

Trans-esterification, a conventional process for producing biodiesel, is normally carried out after oil extraction and refining process [4]. However, the oil contained in microalgae cannot be easily extracted for further processing into biodiesel due to the fact that microalgae is not a simple triglyceride as in palm oil and the likes. Therefore, it is necessary to develop a biodiesel manufacturing process that is simple, efficient, energy efficient and able to produce high-quality biodiesel through the in-situ trans-esterification process. The in-situ method is one of the methods applied in the process of making biodiesel by direct extraction from sources of raw materials containing oil or fat. In the in-situ process the raw material used is solid material containing oil.

Microwave irradiation is one of the new technologies that various processes for the extraction of essential oil from the plant [5] and trans-esterification reactions for the manufacture of biodiesel [6]. In the case of microalgae, microwave irradiation can be used in extracting lipids then followed by trans-esterification reactions to produce biodiesel [7], or simultaneous process extraction and transesterification [8]. It has been reported that microwaves are a simple and effective technology for extracting fat microalgae. Microwave heating can help speed up extraction and at the same time convert it to FAME [9]. Microwave irradiation has been applied in the trans-esterification of oil Chlorella vulgaris [10]. According to this study, using a microwave can reduce reaction time and save energy, as has been explained in several other studies [11].

The purpose of this research is to study the making of biodiesel (methyl ester) through the process of trans-esterification of in situ using microalgae Chlorella sp. as the main ingredient by studying the effects of acid catalyst concentration, microwave power and reaction time. To minimize the number of runs, the experiment was designed and optimized with the response surface methodology using the Behnken Box Design (3 factors and 2 levels).

2. Material and methods

2.1. Material and experimental apparatus
Materials used include microalgae powder Chlorella sp., which was collected from Situbondo Jawa Timur. Other ingredients such as Methanol 96 %, H2SO4 98% etc. are used from MERCK products. In situ trans-esterification was carried out in the Electrolux EMM2308X microwave unit. Biodiesel equipment from microalgae consists of vacuum pumps, condensers, magnetic stirrers, and pyrex reactors. The temperature is controlled using temperature controller equipped with a thermocouple.

2.2. Experimental procedure
In-situ trans-esterification was carried out in a round neck flask, adding 10 gram microalgae powder and 50 ml methanol for all experiments. Microalgae and methanol powder are mixed in round neck flasks. In-situ trans-esterification was carried out at a microwave power of 300 - 600 watts, with a reaction time of 30-90 minutes and catalyst concentrations of 0.2 – 0.5 M. The reactants are then cooled to room temperature and separated by a vacuum filter so that the filtrate and residue are formed. The residue was dissolved in a mixture of hexane and methanol to get the remaining FAME. Ethyl acetate is added to the filtrate to form three layers. The top layer is heated to a temperature of 77.1 °C. Raw biodiesel is then analyzed by gas chromatography (GC).

3. Results and discussion

3.1. The effect of reaction time and microwave power

The microwave power relates to the temperature rise and stability. The temperature instability is found to take place mostly at the microwave power of 300 watt. According to the experiment, 600 watt of microwave power gave the highest reaction temperature compared to that of 300 and 450 watt, respectively. The temperature rise would help break the cellular wall of microalgae, however, excessive use of power and heating causes an overheated reaction that leads to some oil getting burnt [12]. Furthermore, at higher temperatures methanol will evaporate and can interfere with trans-esterification [13]. In addition, the three graphs above have almost the same trend. At 300 watts, the yield of raw biodiesel increases in the reaction time range of 30-90 minutes. At 450 watts, the yield of raw biodiesel increases in the 30-60-minute reaction time range. At 600 watts, the yield of crude biodiesel increases in the 30-60 minute reaction time range and at catalyst concentrations of 0.35 and 0.5 M while the yield of raw biodiesel increases in the 30-90 minute reaction time range and 0.2M catalyst concentration. The highest yield of raw biodiesel is 65%, which was obtained at 450-watt microwave power and 60 minutes reaction time.

Table 1. Experimental results for various variables.

Power (W)	Catalyst concentration (mol/L)	Reaction time (min)	Yield (%)
300	0.2	60	54.51
300	0.35	30	15.88
300	0.35	90	37.83
300	0.5	60	31.98
450	0.2	30	32.44
450	0.2	90	58.02
450	0.35	60	62.51
450	0.5	30	43.30
450	0.5	90	44.47
600	0.2	60	50.67
600	0.35	30	20.06
600	0.35	90	48.43
600	0.5	60	48.72
450	0.35	60	62.51
450	0.35	60	51.23

3.2. The effect of catalyst concentration and reaction time

In addition, Figure 1b shows the effect of reaction time (30-90 minutes) on the results of raw biodiesel on the concentration of H_2SO_4 acid catalyst 0.2 - 0.5 M and effective microwave power of 450 watts. Acid catalyst concentration and reaction time have varied effects on in situ trans-esterification. At a reaction time of 30 minutes, the yield of raw biodiesel increases with higher catalyst concentration. At reaction times 60, 90, and 120 minutes, and catalyst concentrations of 0.35M and 0.5M, the yield of raw biodiesel is lower than the catalyst concentration of 0.2M. This occurs because of the negative effects of high concentrations of acid catalysts that can cause a side reaction called alcohol dehydration.

3.3. Fitting parameters

It can be seen from the Table 2 in ANOVA with the model is generally significant with R^2 of model is 0.9239. The **Model F-value** of 6.74 implies the model is significant. There is only a 2.45% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case C, A^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required

to support hierarchy), model reduction may improve your model. The **Lack of Fit F-value** of 1.02 implies the Lack of Fit is not significant relative to the pure error. There is a 53.09% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The parameters are microwave power (A), concentration of calayst (B) not significant with ($p > 0.05$), but trans-esterification time (C) is significant with ($p < 0.05$). In addition, the quadratic model produces the value of the interaction of each factor. The interaction between all variables the microwave power, concentration of calayst and reaction time (AB, AC, BC) was not significant ($p > 0.05$) which means that the factors not could influence the trans-esterification reaction process. The quadratic of microwave power (A^2) and trans-esterification time (C^2) are significant model terms ($p < 0.05$) means that Quadratic regression model is a good choice. In RSM, p-value of lack-of-fit, ($p > 0.05$) (not significant) means that the model fits well and there are valid and logic model for output responses. Therefore, the results of the all interaction of various factors can be used as a match between experimental data and prediction models. In addition, the another criteria is F-value of 102.46 that implies the model is significant. There is only a 0.25% chance that an F-value this large could occur due to noise. The p-values less than of 0.0500 indicate model terms are significant.

Table 2. ANOVA for Quadratic model to identify significant factors affecting yield.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2597.47	9	288.61	6.74	0.0245	significant
A-Power	95.86	1	95.86	2.24	0.1947	
B-Catalyst	92.30	1	92.30	2.16	0.2019	
C-Time	742.74	1	742.74	17.36	0.0088	
AB	105.91	1	105.91	2.47	0.1765	
AC	10.32	1	10.32	0.2411	0.6442	
BC	148.90	1	148.90	3.48	0.1211	
A^2	638.05	1	638.05	14.91	0.0119	
B^2	2.76	1	2.76	0.0645	0.8096	
C^2	836.74	1	836.74	19.55	0.0069	
Residual	213.98	5	42.80			
Lack of Fit	129.19	3	43.06	1.02	0.5309	not significant
Pure Error	84.78	2	42.39			
Cor Total	2811.44	14				

The fit summary and statistic show that is confirm for the use of the model. After optimization, the model is used to produce the proper response. The model shows various interaction relationships between several parameters used. In table 4 shows the difference between adjusted R^2 ($R^2 = 0.9828$) and predicted R^2 ($R^2 = 0.9369$) is less than 2%, is in reasonable agreement. Therefore, it could be concluded that second-order model is used in extraction optimization from production of methyl ester from micro algae oil using Sulfuric acid.

3.4. Analysis interaction parameters of Box-Behnken design

A total of 17 experiments were carried out to estimate the response surface the biodiesel yield. The response variable is that the biodiesel yield (Y) depends on the independent variables realize through the experiments is indicated in table 1. The results showed that there is a contact between factors biodiesel yield. To determine which of the effects in the model are statistically significant, the p-value of regression was significant with a commonly used α-level of 5% [14,15]. Moreover, responses greater than 0.10 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The quadratic model is described by the following as in Eq. (2):

Yield (%) = 58.75+3.46A-3.39B+9.64C+5.15AB + 1.61AC - 6.10BC - 13.15A^2 + 0.86B^2 - 15.05C^2

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Figure 1 shows a 2D model of the relationship between the attained biodiesel yield and three independent factors: microwave power (A), concentration of catalyst (B), and trans-esterification time (C). This indicates that fixed variables (microwave power (A), concentration of catalyst (B), and trans-esterification time (C) have an effect on the model. Based on figure 1 (a), it could be seen that microwave power (A) affect the extraction yield, interaction between microwave power and concentration of catalyst (AB). Based on optimised, the optimal condition are microwave power at 400 W and concentration of catalyst in 1.5%.

Figure 1(b) showed the interaction between microwave power and trans-esterification time (AC). In the contour response, the optimum conditions are very near 400 W and 3 min from experimental data. Surface plots proved that the microwave power and trans-esterification time are as same as sensitive to produce the yield. As shown in Figure 1 (c) could be seen by the amount of concentration of catalyst will reduce the existing results. Besides that, the higher of trans-esterification time would be greater result of the extraction yield.

Therefore, the desirability function from this optimization is 1.00. Furthermore, in the study from trans-esterification in the production of methyl ester from micro algae oil using acid catalyst was to evaluate the effect of parameters using microwave-assisted technique. Based on the second-order model, the optimum conditions calculated for optimization of the reaction of trans-esterification when microwave power (300-600W), concentration of catalyst (0.2 – 0.5M), and trans-esterification time (30-90 min). It was chosen to minimize the standard error of yield. While the results of the experimental data to obtain the highest yield conditions at (370 W; 0,2 M and 82.7 min) is 63.36 %, which is very close to the predicted value of 63.36 %. This indicates that the model is accurate to predict the expected optimal. Besides, the error rates between the experimental and predicted model are less than 5%. Therefore, the extraction process from trans-esterification for any parameters, such as microwave power and trans-esterification time, could be accurately predicted by the regression models designed by response surface methodology.
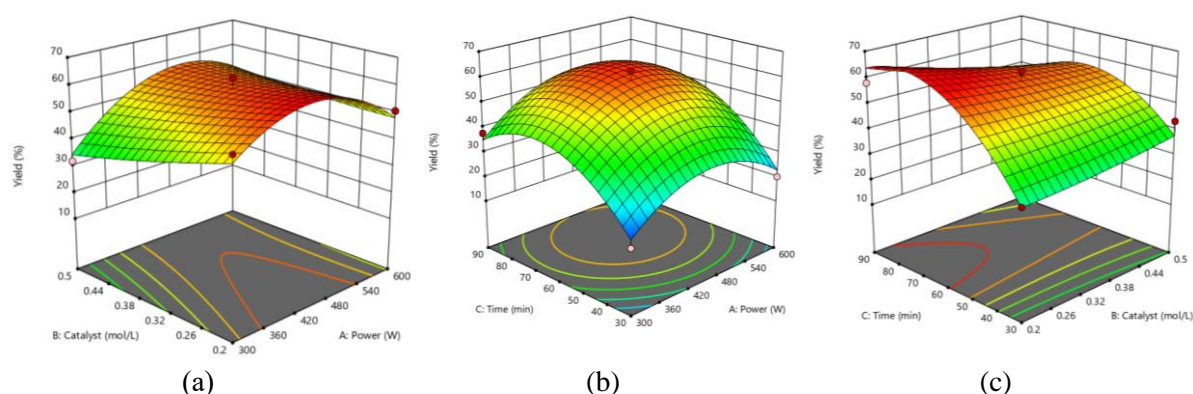

(a)   (b)   (c)

Figure 1. 2D Plots representing parameters influencing yields of trans-esterification in the production of methyl ester from micro algae oil using Sulfuric acid (**a**) effect microwave power and concentration of calayst; (**b**) effect of concentration of calayst and trans-esterification time; and (**c**) effect of concentration of calayst and trans-esterification time.

4. Conclusion
The optimization performed by Box-Behnken design, the conditions for trans-esterification of nyamplung oil using microwave with acid sulfuric catalyst have been suggested as follows: microwave

power (370 W), concentration of calayst (0.2 M) and trans-esterification time (82.7 min) with yield of 63.6 %. Box-Behnken design and trans-esterification of nyamplung oil using microwave with aci catalyst showed not only the convenience of the experiments, but also the optimization of the three parameters in detail, which made the yield of biodiesel obtained more efficiently both quantity and quality.

Acknowledgment

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