Optimization microwave assisted transesterification insitu for biodiesel production from *Chlorella* sp. using response surface methodology

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**Abstract.** *Chlorella* sp. is a potential raw material for biodiesel production because of its high lipid content. Microwave assisted transesterification insitu from *Chlorella* sp. was investigated to determine the effect of reaction time, catalyst concentration and microwave power on biodiesel yield using response surface methodology for optimization. In this process the use of microwave irradiation to speed up the lipid extraction process and the transesterification reaction is simultaneously converted to methyl-esters. Faced-centered Central Composite Design (FCCD) design was applied to evaluate the effects of three independent variables: reaction time (10-50 minutes), microwave power (300-600 W), and H₂SO₄ catalyst concentration (0.1-0.9 gr/mL). The results showed that biodiesel yield increased with reaction time, power and catalyst concentration. Through optimization with response surface, this methodology with Face-Center Central Composite (FCCD) gave the highest biodiesel yield of 19.5% at 561 W, 40.5 minutes and 0.42 gr/mL catalyst concentrations with p-values from lack of fit insignificance, indicating that the model was a good representation of experimental.

**Keywords:** Biodiesel, FCCD design, Chlorella sp., microwave assisted transesterification

1. Introduction

Searching for renewable and clean energy now is very important to meet global energy needs for three reasons, those are the decline in fossil energy reserves, world population growth and environmental problems due to exhaust gases from combustion [1,2]. At present, biodiesel has attracted public attention as one of the best renewable and environmentally friendly energy to replace diesel oil from petroleum [2,3]. Conventionally, biodiesel is produced from first-generation vegetable oils such as palm oil, coconut, soybean, corn, sunflower, peanuts, and others. These oils are edible so that they compete with food need. Biodiesel also produced from second-generation vegetable oils such as vegetable oil, castor oil, and nyamplung oil. These oils are not edible, but their cultivation requires large land. [3,4,5]. Besides, the main constraints of this conventional raw material are relatively high prices and unsustainable supplies [6,7]. Whereas for third-generation biofuel feedstocks, microalgae-based biodiesel has been suggested as one of the most promising alternatives to fossil fuels [8] because of its potential benefits. Those are higher biomass productivity, faster growth rates, and higher content.
of lipids [8,9]. Also, microalgae have other environmental benefits because of their photosynthetic activities, such as CO₂ mitigation and bioremediation of wastewater.

Various types of microalgae have been developed such as Nannochloropsis, Chlorella sp., Spirulina sp., Chlorella vulgaris or Arthrospira platensis, Haematococcus pluvialis, and Phaeodactylum tricornutum, as a source of natural dyes, medical substances and as a source of protein that has been used for the food industry [10]. Whereas in East Java microalgae farmers develop Chlorella sp. because of easy cultivation and relatively high lipids.

The current transesterification method commonly used in the biodiesel industry uses various raw materials for vegetable oils such as palm oil, rapeseed oil, sunflower oil, etc. Whereas in making biodiesel from microalgae there are two possible paths. The first route is lipid extraction followed by the conversion of lipids into fatty acid methyl esters (FAME) and glycerol. This pathway certainly requires a higher cost and time [11]. In addition, the use of solvents for extraction may involve expensive separation processes. Whereas the second alternative route is the insitu transesterification process. This process has the potential to reduce the unit cost of the extraction process and the cost of the biodiesel conversion process, because the lipid extraction process and the conversion of lipids into biodiesel occur simultaneously in one single reactor [12]. The transesterification method insitu seems to be the most economical compared to several other methods that have been applied including extraction followed by transesterification reaction, biochemical methods (fermentation, and anaerobic digestion) and thermochemical methods (combustion, gasification, liquefaction, and pyrolysis).

Recently, the insitu process has been implemented using various heating processes such as conventional heating, ultrasonic, and microwave heating. Martinez-Guerra, et al 2014 [13] reported to compare the use of these heating and the results showed that the use of microwaves was more efficient because of the smallest energy consumption compared to the others (microwave (26 MJ/kg) and USG (44 MJ/kg) [13]. This is because the ability of microwaves to penetrate the cell wall structure can produce efficient oil and fat recovery [7].

Another technical consideration in making biodiesel is the application of catalysts. Several researchers [14,15] examined the use of two catalyst groups for the conversion of microalgae oil, namely strong acid catalysts (HCl, H₂SO₄) and strong alkalis (KOH, NaOH). Some of these authors demonstrate some of the advantages of using an acid catalyst compared to an alkaline catalyst for the production of fatty acid methyl esters (FAME) from microalgae oil [14].

Therefore, in this study, the conversion of microalgae Chlorella sp. using transesterification of insitu assisted microwaves using H₂SO₄ acid catalyst was studied. The purpose of this study was to determine the effect of microwave irradiation, reaction time, concentration of acid catalysts in producing biodiesel. The response surface methodology using the FCCCD was applied to obtain optimal conditions of the effect of parameters in the process of making biodiesel by insitu transesterification assisted by microwave.

2. Material and Methods

2.1. Materials and Experimental Apparatus

Chlorella sp. powder was used in this research. It was obtained from the Brackish Aquaculture Center (BBAP) in Jepara, Central Java. The catalyst in this study was sulfuric acid (H₂SO₄). While methanol, the reagent for transesterification, was obtained from the chemical supplier PROLAB-UN 1230 Brataco Chem brand Made in France with 99% content and was assisted with n-hexane solution (96%) as a solvent and co-solvent in the process.

The main equipment used in this study was an Electrolux microwave EMM2007X model with a frequency of 2.45 GHz and a maximum power of 800 W. The transesterification reaction was carried out in a three-neck pumpkin glass reactor equipped with a condenser.
2.2. *Experiment Procedure*

The research was carried out by dissolving the catalyst into methanol according to the specified variable and stirring it with a magnetic stirrer. The methanol and catalyst mixture were inserted into the reactor containing 20 gr microalgae powder. It was put in the microwave then the system was turned on according to the predetermined variable. This process was carried out on all variables of the microalgae-methanol ratio, catalyst concentration and microwave power. After the reaction process was complete, the mixture was filtered using a vacuum filter to separate the filtrate. The mixture was resuspended with methanol for 10 minutes to remove the remaining free acid methyl ester (FAME) and then the obtained filtrate was cooled. Water was added to the filtrate solution to facilitate the separation of the hydrophilic components before being separated, pushed apart for 3 to 3 layers formed. A number of FAME in the first layer formed were extracted 3 times with n-hexane solution and washed with water to remove the remaining catalyst and methanol. The FAME product obtained then was distilled to remove the remaining hexane and then was weighed. The methyl ester product obtained was determined by the yield and was analyzed by gas chromatography-mass spectroscopy.

2.3. *Experiment Design*

Design and analysis of experiment for optimization of process parameters were performed on Design Expert 12 with the design type is faced-center central composite design (FCCD). Microwave power (A, 300–600 W), H$_2$SO$_4$ concentration (B, 0.1–0.9 gr/mL) and reaction time (C, 10-50 min) were chosen as the independent variables with 3 level for each factor and can be seen in Table 1.

| Factor          | Unit | Level     |
|-----------------|------|-----------|
|                 |      | Low       | Middle | High   |
| A: Microwave power | W    | 300       | 450    | 600    |
| B: Catalyst time | g/mL | 0.10      | 0.50   | 0.90   |
| C: Reaction time | min  | 10        | 30     | 50     |

After entering each variable in the design expert with 5 replicates at the center points leading to a total number of 20 experiments and are shown in Table 2. Biodiesel yield (\%) was the dependent output variable.
### Table 2. Summary of experiment design

| Run | Coded Value | Response: Yield (%wt) |
|-----|-------------|----------------------|
|     | Micro. power (watt) | Catalyst conc. (mL/gr) | Reaction time minute |     |
| 1   | -1          | -1                    | -1                   | 3.00  |
| 2   | -1          | -1                    | +1                   | 26.32 |
| 3   | -1          | 0                     | 0                    | 16.39 |
| 4   | -1          | +1                    | -1                   | 7.67  |
| 5   | -1          | +1                    | +1                   | 23.41 |
| 6   | 0           | -1                    | 0                    | 15.78 |
| 7   | 0           | 0                     | -1                   | 10.36 |
| 8   | 0           | 0                     | 0                    | 20.06 |
| 9   | 0           | 0                     | +1                   | 21.33 |
| 10  | 0           | +1                    | 0                    | 30.92 |
| 11  | +1          | -1                    | -1                   | 10.10 |
| 12  | +1          | -1                    | +1                   | 22.47 |
| 13  | +1          | 0                     | 0                    | 13.10 |
| 14  | +1          | +1                    | -1                   | 23.1  |
| 15  | +1          | +1                    | -1                   | 25.31 |
| 16  | 0           | 0                     | 0                    | 18.51 |
| 17  | 0           | 0                     | 0                    | 20.17 |
| 18  | 0           | 0                     | 0                    | 24.44 |
| 19  | 0           | 0                     | 0                    | 18.11 |
| 20  | 0           | 0                     | 0                    | 17.77 |

Code factor formulas can be used to make predictions about the response to the level given by each factor. Where Y is predicted responses (Yield), A, B, C are independent variables which respectively indicate microwave power (W), acid concentration H₂SO₄ (gr/mL) and reaction time (min). By default, high levels of factors are coded +1 and low levels are coded -1. Hence, lowest levels are coded -1, middle values are coded 0, and highest levels of factors are coded +1. Code equations are used to identify the relative impact of factors by comparing the factor coefficients in Equation (1).

Coded value was finished to know the description of the individual independent variables effect on the response. The dependency of each experimental response is symbolized by Y and the model as follows:
\[ y = \beta_0 + \sum_{i=1}^{n}\beta_i x_i + \sum_{i=1}^{n}\beta_{ii}x_i^2 + \sum_{i=1}^{n}\sum_{j=i+1}^{n}\beta_{ij}x_i x_j + \varepsilon \]  

(1)

Where \( \beta_0 \) is a term constant, \( \beta_i, \beta_{ii}, \beta_{ij} \) are coefficient, \( \varepsilon \) is error factor, \( x_i \) and \( x_j \) is variable (A, B and C) and \( n \) is amount of variable. The coefficient is determined by multiple linear.

3. Results and Discussion

3.1 Microwave Assisted Transesterification Insitu

The well-known process for converting triglycerides or lipids into methyl esters is the transesterification method. This method involves alcohols of short alkyl groups, such as the reaction of methanol with a long chain of fatty acids (triglycerides) and carried by an alkoxide anion, which is facilitated by the presence of an alkaline catalyst [16]. But, development of biodiesel production in conventional process from biomass involves two steps: extraction and transesterification that requires much energy, high operational cost and need larger solvent so it is not efficient [17]. Transesterification insitu of microalgae is a transesterification process that occurs directly (direct transesterification) or simultaneously in producing biodiesel. This process is reported to be superior to two-stage transesterification or conventional processes that is more dependent on extraction results with organic solvents with low separation efficiency because of the incomplete extraction process. The ineffective use of solvents, the length of time and the many stages of the process are also things that cause high operational costs and product prices. In this study the transesterification of insitu microalgae in producing biodiesel was studied using a microwave as a heater. Using microwave is intended to replace ineffective conventional heating systems in terms of energy and time saving. Energy efficiency of heating by using microwave is much smaller when compared to conventional heating because the rapid heating causes localized temperature and high-pressure differences which cause degradation of the cell wall thereby accelerating the rate of mass transfer [7].

3.2 Development of Regression Model

Using Design Expert Software version 12.0, analysis of variance (ANOVA) based on the face-centered central composite design (FCCD) for the quadratic model is obtained in Table 3. From the ANOVA shows that three parameters have a significant effect on biodiesel yield, which is indicated by the significant P-value. The P-value of the mismatch is 0.0020 and the model F-value of 7.59, which is not more than 0.05 (95% confidence level) indicating the curriculum model provides an accurate description of the experimental data which shows the success of the three parameters of the transesterification process that produces biodiesel from algae. In addition, it is supported by a large enough conversion coefficient value, \( R^2 \) (0.87).
Table 3. Analysis of variance of design for response surface quadratic model for methyl ester content

| Source   | Sum of Squares | df | Mean Square | F-value | p-value | significance |
|----------|----------------|----|-------------|---------|---------|--------------|
| Model    | 791.40         | 9  | 87.93       | 7.59    | 0.0020  | significant  |
| A-Power  | 28.10          | 1  | 28.10       | 2.43    | 0.1503  |              |
| B-Catalyst | 89.82         | 1  | 89.82       | 7.76    | 0.0193  |              |
| C-Time   | 284.00         | 1  | 284.00      | 24.53   | 0.0006  |              |
| AB       | 23.02          | 1  | 23.02       | 1.99    | 0.1889  |              |
| AC       | 47.54          | 1  | 47.54       | 4.11    | 0.0703  |              |
| BC       | 25.01          | 1  | 25.01       | 2.16    | 0.1724  |              |
| A²       | 37.85          | 1  | 37.85       | 3.27    | 0.1007  |              |
| B²       | 63.52          | 1  | 63.52       | 5.49    | 0.0412  |              |
| C²       | 18.90          | 1  | 18.90       | 1.63    | 0.2303  |              |
| Residual | 115.79         | 10 | 11.58       |         |         |              |
| Lack of Fit | 83.00      | 4  | 20.75       | 3.80    | 0.0716  | not significant |
| Pure Error | 32.79        | 6  | 5.47        |         |         |              |
| Cor Total | 907.19        | 19 |             |         |         |              |

Std dev. 3.40, R² (the regression coefficient) 0.8724, adj. R² 0.7575, pred. R² -0.3380, adequate precision 10.4189

The regression coefficient (R²) shows and measures goodness of fit for the regression model. R² must be high for smaller differences between actual data and prediction data. So, if R² is greater than 80%, the model is said to be appropriate. This study has an R² of 87.24% (0.8724) so this model can be used and a negative predicted R² implies that the overall average might be a better predictor of your response than the current model. In some cases, higher or lower order models can also predict better. While adequate precision measures the ratio of signal to noise. A ratio greater than 4 is desired. The calculation results show adequate precision of 10.419, this shows an adequate signal, so this model can be used to represent experimental data properly [18].

![Predicted vs. Actual](image.png)

**Figure 1.** Predicted Chlorella sp. methyl ester yield versus experimental yield
Figure 1 shows the plot between experimental yield of Chlorella sp. methyl ester and those predicted from the model. From this figure shows that the experimental and predicted values are almost the same with each other. This shows the accuracy of the model in correlating biodiesel yield with independent reaction variables. Thus, the quadratic model chosen can represent the experimental data well.

3.3 Effect of Parameters and Optimization

The reaction parameters that affect biodiesel yield during an insitu microwave-assisted transesterification reaction are microwave power, catalyst concentration and reaction time. To show the effect of these variables on the dependent variable, a contour plot is made. Figure 2 shows the response surface plot to connect the three parameters used in this study and these parameters are considered as the most significant factors influencing the yield of methyl esters.

![Contour plots](image)

**Figure 2.** 2D and 3D contour plots presenting the effects of the microwave power (A), catalyst concentration (B), reaction time (C) on the biodiesel yield of microalgae using microwave-assisted transesterification insitu with FCCD

The results showed that methyl ester of Chlorella sp. yield increased with reaction time, microwave power and catalyst concentration. Using a quadratic model from Equation 1 and numerical optimization of response surface methodology, the maximum yield is 19.5% at microwave power 561 W, 40.5 min and 0.42 gr/mL H₂SO₄ concentrations and can be seen highlighted in response surface methodology plots as shown in figure 2.

Figure 2 (left side) shows relation between microwave power and catalyst concentration (H₂SO₄) with the methyl ester of Chlorella sp. yield (%). Based on the plot, it appears that microwave power can increase the yield where the maximum yield is obtained at the range power of 380-600W as the concentration of H₂SO₄ increases (0.8-0.9 gr/mL). The high power of the microwave enhanced
methanol dipole moment and destroys the boundary between the oil and methanol phases. This mechanism reveals higher biodiesel yields at high power for short reaction times [19]. Furthermore, microwave power becomes a driving force and produces heating localization to facilitate disrupting the matrix of microalgae, therefore the solute which in this case is triglycerides or methyl ester is easier to diffuse and dissolves in the solvent. In addition, energy is transferred by microwaves into the material through dipolar polarization, ionic conduction and interface polarization which causes localization of heating to the material and accelerates the transesterification reaction [20]. However, increasing the power beyond 600W does not further increase the conversion of yield probably due the destruction of the biodiesel components. After that, figure 2 (center side) shows relation between reaction time (min) and microwave power (W) on the yield (%). Based on the contours above, the maximum yield obtained by red contours in the reaction time range above 40 min to 50 min with a power range of 380-580W. This result is consistent with the results of numerical optimization of RSM which states that the maximum yield obtained at a reaction time of 40.5 min at 561 W of power usage and yield will be reduced with additional reaction time. Methyl ester of Chlorella sp. yields drop significantly after a certain reaction time period because it triggers a reversible reaction. The introduction of the two-stage reaction process was found to eliminate the reversibility process which leads to better results [16]. In this result, it can be concluded that the use of the longer reaction time will increase the yield value of biodiesel.

In addition, figure 2 (right side) shows relation between catalyst concentration (H2SO4) and reaction time (min) on yield (%). The contours of the image describe that the maximum yield (red contour) is obtained when using catalyst in the range of 0.6-0.9 mL/gr with a reaction time range of 30 - 50 min. Catalysts provide an important role in increasing biodiesel yield and the dosage of catalyst has its optimal level in transesterification. Generally, when catalyst has reached the optimal level, catalyst will be inefficient and maximum yield cannot be reached. However, excess catalyst can also result in saponification reaction [21]. This optimization gives the result that the optimum catalyst is 0.89 mL / gr to obtain maximum yield. Moreover, the excessive catalyst amount cause to the gel or slurry formation with increased viscosity of solution mixture. This effect is probably responsible for disturbance mixing of solution constituents leading to decreased yields with further addition of catalyst [22].

4. Conclusion
The results showed that biodiesel yield increased with reaction time, power and catalyst concentration. Through optimization with response surface, face-center central composite design (FCCD), gave the highest biodiesel yield of 19.5% at 561W, 40.5 mi and 0.42 gr/mL. Catalyst concentrations with P-values from lack of fit insignificance, indicating that the model was a good representation of experimental.

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6. Acknowledgment

This research is supported by research funding from the DRPM-RISTEK-DIKTI of the Republic of Indonesia through the Higher Education Excellence Basic Research (PDUPT) scheme.