

ORIGINAL RESEARCH ARTICLE

Response surface methodology on enzymatic interesterification of *Calophyllum inophyllum* seed oil using immobilized *Rhizomucor miehei* lipase to produce biodiesel

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ABSTRACT

Biodiesel is among the renewable and clean energy to substitute diesel fuel. One of the prospective raw materials for biodiesel is nyamplung (*Calophyllum inophyllum*) seed oil. Biodiesel production generally applies transesterification reaction with methanol assisted by inorganic base catalyst to produce fatty acid methyl esters with the by-product glycerol. To eliminate glycerol by-product, methanol can be replaced with ethyl acetate which results in triacetin byproduct. Triacetin can increase the cetane number, hence it is not necessary to remove it from biodiesel product. The use of conventional catalysts in biodiesel production produces chemical waste and requires high purification costs. Enzyme catalysts such as lipase is an alternative to overcome these problems. However, lipase is costly and sensitive with the changing of operation condition. To cope with this issue, lipase is immobilized into solid material. In this work, interesterification of nyamplung oil with ethyl acetate using immobilized lipase was conducted. Optimization using response surface methodology (RSM) was also performed. Based on the RSM analysis, it was shown that the optimum conversion conditions were 47.40% with selectivity of 14.77% and a yield of 6.32% at a temperature of 50°C, with a molar ratio of 1:8.99 and an immobilized lipase catalyst concentration of 7.49%.

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1. Introduction

Fuel consumption in the world is increasing significantly. Currently, the fuel used comes from non-renewable resources such as petroleum, natural gas and coal. Therefore, alternative renewable fuels are needed to reduce the use of fossil fuel. Biomass is the potential renewable resource for biofuel production in the future, such as

biodiesel, bioethanol, bio-oil, and syngas^[1-3]. The key conversion technology to transform biomass to biofuel are biochemical, thermochemical, and chemical conversions. Biodiesel is one of the most applicable biofuels since it has many superior characteristic, i.e. biodegradable, sustainable, and harmless^[4].

Biodiesel is an alternative energy resources produced from vegetable oil as raw material. Various raw material can be used as feed-stock for making biodiesel such as palm, jatropha, rubber seeds, nyamplung (*Calophyllum inophyllum*) seeds oils, etc^[5-7]. Apart from vegetable oils, animal fat, and used fats can also be used for biodiesel because they also contain triglycerides. Of several raw materials, nyamplung seed has good potential for processing into biodiesel because their oil yield content is relatively high at 40-70%. It is higher than the oil content of other plants, for example oil palm, jatropha curcas, and rubber seeds are at 46-54%, 40-60%, 40-50%, respectively. In Indonesia, nyamplung plants are often found on the coast and are easy to cultivate.

Biodiesel production generally conducted via transesterification reaction of vegetable oil with methanol in the presence of inorganic alkaline catalysts such as sodium or potassium methylate^[8]. This reaction yields a mixture of fatty acid methyl esters with the by-product glycerol. The formation of glycerol by-product leads to the increasing production cost since glycerol separation from biodiesel product is obligatory. To eliminate glycerol generation, methanol can be replaced with ethyl acetate. The reaction between vegetable oils and ethyl acetate is called interesterification process, which results in triacetin as by-product. Triacetin can increase cetane number^[9], in such a way that separation of by-product from biodiesel is not necessary.

The use of conventional alkaline catalysts in biodiesel production apparently produces chemical waste that is harmful to the environment and requires high product purification costs. Moreover, the presence of alkaline catalyst is not suitable for feedstocks containing high free fatty acid (FFA) since undesired saponification reaction will occur. The FFA should be remove via pre-esterification reaction prior to transesterification process. Instead, heterogenous catalyst can be employed to eliminate the drawback of homogeneous catalyst. The most popular heterogeneous base catalyst is calcium oxide on account of its excellent catalytic activity^[10].

The other choice is the usage of biocatalyst. Application of biocatalyst which is environmentally friendly becomes an attractive alternative to solve these drawbacks. Lipase enzyme is among biocatalyst which can be employed for interesterification of vegetable oils to produce biodiesel^[11]. However, utilization of liquid lipase catalyst in industrial process faces a challenge since it is expensive and unstable. It has limitation in recovery and reusability, as well^[12]. To defeat this issue, lipase enzyme can be attached to a support such as insoluble solid carried, carrier matrix, and so forth. Lipase is tied up to the support by reversible physical adsorption and ionic bonds to fix covalent connection, delivering catalytic and thermal stability. This method known as immobilized lipase. Immobilized lipase is likewise more resistant to denaturation, with the result that it increases its reusability^[13,14]. In this work immobilized lipase on immovead from *Rhizomucor miehei* catalyst was applied to catalyze interesterification of nyamplung seed oil with ethyl acetate to produce biodiesel.

To obtain the highest reaction conversion, optimization using Response Surface Methodology (RSM) was also performed in this study. RSM is a statistical method which can be utilized to improve and optimize process. The response in RSM is influenced several factors of independent variables. There are various experimental design models used in RSM, namely Central Composite Design (CCD), Box-Behnken Design (BBD)^[15], design mixture/ D-optimal^[16], Fractional Factorial Design^[17], Simplex Lattice Design^[18], Uniform Design, and others. Response Surface Methodology (RSM) analysis is useful in determining operating conditions to achieve the desired conversion Besides, there are also other statistical methods such as Plackett-Burman Design^[19] and Taguchi^[20].

Central Composite Design (CCD) includes a combination of central points and corner points to better describe the response surface. This design allows the identification of linear and non-linear relationships

between input factors. Box-Behnken Design (BBD) focuses on identifying the relationship between input and output factors without having to involve too extreme angles. This can help in avoiding extreme problems in experiments, while still providing relevant information. Fractional Factorial Design is useful when the input factors have such complex interactions. In fractional factorial design, partial combinations of factors are tested to save time and resources. Plackett-Burman Design is used to identify the important factors that most influence the response, and on the other hand, minimizing the number of trials required. In D-optimal Design, the test points are selected to provide maximum accuracy and efficiency in building the response model. Taguchi Design is more commonly used in Quality Engineering, but it can also be applied in RSM to optimize response by considering variations and noise factors. Simplex Lattice Design is suitable for situations where there are limitations in the range of input factor values. Uniform Design is a method for determining a combination of test points that is even and efficient throughout the design space^[21].

Among the methods, BBD is efficient and practical since it has extreme points and requires. BBD is more efficient and cost-effective than DD and CCD since it has no extreme points and needs a smaller number of points than the others for the analysis and optimization. Therefore, in this study, the main variables are optimized using BBD to obtain the highest yield of biodiesel in the interesterification reaction of nyamplung seed oil with ethyl acetate in the presence of immobilized *Rhizomucor miehei* lipase catalyst. This work also supports the achievement of the SDGs 7, affordable and clean energy.

2. Materials and methods

2.1. Materials

Material used in this work were crude nyamplung (*Calophyllum inophyllum*) seed oil from UMKM Samtamanu, Cilacap, Indonesia, ethyl acetate p.a. (Merck), immobilized on immovead 150 from *Rhizomucor miehei* (Sigma), phosphoric acid p.a (Merck), KOH p.a (Merck), oxalic acid p.a (Merck), ethanol p.a (Merck), n-hexane p.a (Merck), and distilled water from local chemical store.

2.2. Methods

2.2.1. Degumming of Nyamplung Seed Oil

Nyamplung oil contains high gum and has dark color so that degumming of the oil prior to the main interesterification process is necessary. Degumming process was conducted through the following procedure. Initially, 250 mL of nyamplung seed oil was put into a 500 mL flat bottom flask, then the oil was heated at a temperature of 70°C. The heating process was performed using a hot plate accompanied by stirring using a magnetic stirrer at a speed of 1000 rpm. The oil was subsequently added with 0.3% (w/w oil) phosphoric acid (0.41 mL) then heated for 25 minutes. Having finished the heating process, the oil was cooled to room temperature. It afterwards was put into a 500 mL separating funnel and left for 24 hours. Distilled water was then added at a temperature of 40°C with a volume of 5% of the oil volume (12.5 mL). After that, degummed oil was separated from the residue and heated in an oven at a temperature of 105°C to reduce the water content. The refined oil was ready to undergo the main interesterification process.

2.2.2. Interesterification of Nyamplung Oil to Produce Biodiesel

The main process interesterification of nyamplung seed oil with ethyl acetate employing immobilized *Rhizomucor miehei* Lipase catalyst was carried out at fix variables as followed: weight of nyamplung seed oil, hot plate rotation speed, and reaction time of 20 g, 600 rpm, and 8 hours, respectively. The independent variables studied in this research were reaction temperature (30°C, 40°C, 50°C), molar ratio of oil and ethyl acetate (1:3, 1:6, 1:9), and catalyst concentration (2.5%, 5%, 7.5%). Firstly, nyamplung seed oil and immobilized lipase were mixed and heated in the three necks flask reactor to a certain temperature. On the other hand, ethyl acetate was also warmed up separately. Once the desired temperature was attained, ethyl

acetate was poured into the reactor and mixed with nyamplung seed oil and immobilized lipase evenly. Interesterification reaction was run for 8 hours. Thereafter, biodiesel produced was separated from the reactant and immobilized lipase residue by using centrifugation at 40,000 rpm for 15 minutes. To determine reaction conversion, selectivity, and biodiesel yield, fatty acid composition of the sample was analysed using Gas Chromatography-Mass Spectroscopy (GC-MS).

2.2.3. Sample Analysis

Fatty acid composition of each sample was discovered using a Gas Chromatography Mass Spectrometer equipment (GC MS-QP2010 SE) with a column flow of 1.20 mL/min, oven column temperature of 65°C, pressure of 74.5 kPa, and injection temperature of 250°C with split injection mode. The internal standard used is heptadecanoic acid, methyl ester. Reaction conversion, selectivity, and biodiesel yield was calculated based on the fatty acid composition obtained. Yield of biodiesel represents the ratio of methyl ester to oil weight. Optimization was then found out according to Response Surface Methodology (RSM) analysis, which was carried out using Design Expert software.

2.2.4. Optimization Using Box-Behnken Design of Response Surface Methodology

Optimization of the operation condition of the enzymatic nyamplung seed oil interesterification was performed using Box-Behnken Design (BBD) of Response Surface Methodology (RSM). The RSM simulation applied Design Expert Software and optimized the operation condition (temperature, molar ratio of reactant, and catalyst concentration) providing the highest reaction conversion, selectivity, and biodiesel yield. BBD was employed since it can optimize the operation parameters with small number of experiments and enables analysis of interaction between the variables. In this work, BBD optimization was carried out using a total of 17 experimental runs, in which the center point measurements were repeated three times to bring about a precise calculation and minimize the effect of experimental error. Variables evaluated in this work were temperature (A), molar ratio of ethyl acetate to oil (B), and catalyst concentration (C). Design of the randomized responses model is presented in **Table 1**.

Table 1. Design of the Randomized Response Model.

Run	Temperature (°C)	Molar Ratio of Ethyl Acetate to Oil	Catalyst Concentration (%)
1	30	6	2,5
2	30	9	5
3	50	6	2.5
4	40	6	5
5	40	3	7.5
6	40	3	2.5
7	50	9	5
8	30	6	7.5
9	40	6	5
10	40	9	7.5
11	30	3	5
12	40	9	2.5
13	40	6	5
14	50	6	7.5
15	50	3	5
16	40	6	5

3. Results and Discussion

3.1. Degumming and Characteristic of Feedstocks

Degumming process of nyamplung oil aimed to remove unwanted gum or phospholipids acid content which could interfere the next process and lower biodiesel product quality. Degumming is the initial step in vegetable oil refining. There are some methods that can be applied for degumming such as water degumming, enzymatic degumming, acid degumming, membrane degumming, and EDTA degumming. Degumming utilizing low phosphoric acid is known as an effective and low-cost degumming process for vegetable oil [22]. In this work, 0.3% phosphoric acid was applied. The comparison of nyamplung seed oil before and after degumming is presented in **Table 2**.

Table 2. Characteristic of Nyamplung Seed Oil.

Properties	Before Degumming	After Degumming
Viscosity (mm ² /s)	58.3325	56.4860
Acid Number (mg KOH/g)	58.043	37.94

Degumming process showed the colour changing of the oil from dark green to reddish yellow. It was due to the removal of phospholipid in the oil. Phospholipids have been found to enhance the colour reversion of oils. Therefore, the reduction of phospholipids content can improve the clarity of the oil [23]. Degumming also decreased the values of viscosity and acid number of nyamplung seed oil as depicted in **Table 2**. The viscosity value decreased by 3.16% and the acid number was diminishing from 58.043 mg KOH/g oil to 37.94 mg KOH/g oil. The lower viscosity occurred since the phosphoric acid bound the viscous gum and other impurities. Meanwhile, the decreasing of the acid number was caused by the depletion of free fatty acid during the degumming process [24]. The degumming process using phosphoric acid can eliminate up to 85.9% gum content [25].

The degumming process removed 85.9% of the phosphorus content from crude kenaf seed oil.

Analysis of the molecular weight of nyamplung seed oil was conducted using GC-MS. GC-MS chromatogram is shown in **Figure 1**. Based on the GC-MS evaluation, the composition of nyamplung seed oil can be revealed as demonstrated in **Table 3**.

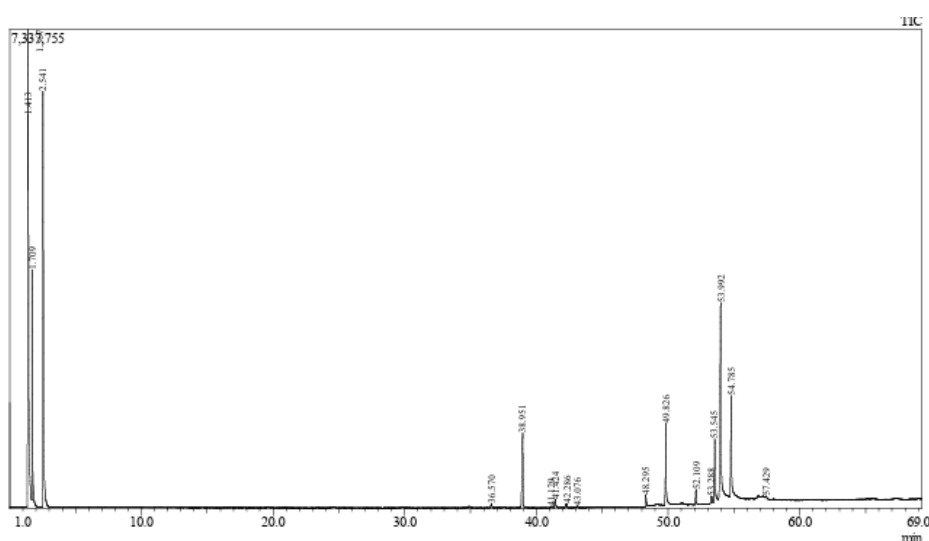


Figure 1. GC-MS Chromatogram of Nyamplung Seed Oil.

Table 3. Composition of Nyamplung Seed Oil.

Fatty Acid	Molecular Weight (g/mol)	Area (%)
Palmitic Acid	262.22	5.14
Linoleic Acid	280.45	7.49
Elaidic Acid	282.46	18.53
Stearic Acid	284.47	4.88
Arachate Acid	312.54	0.16

3.2. RSM Analysis on Immobilized Lipase Catalyst Interesterification

Interesterification reaction was accomplished between nyamplung seed oil with ethyl acetate employing immobilized *Rhizomucor miehei* lipase catalyst to produce biodiesel. The reaction of ethyl acetate with triglyceride resulted in triacetin as by-product instead of glycerol^[26]. The experimental data of the reaction conversion, selectivity, and biodiesel yield was calculated by determining fatty acids concentration using GC-MS. Biodiesel yield represents the ratio between weight of biodiesel produced to the weight of oil^[27]. Thus, it describes how much the feedstock was consumed. Conversion reaction formulated as the ratio between the oil reacted to the total oil introduced to the reactor. Thus, it shows how much raw material was used in the reaction. In biodiesel production, conversion is also determined as the consumption of triglycerides in reaction. Selectivity shows the efficiently the oil converted to ester.

The data was analyzed using Response Surface Methodology (RSM) for optimization. In this study, design of operation condition was assessed with the Box-Behnken Design (BBD) in RSM to optimize independent variables (molar ratio of ethyl acetate to oil, reaction temperature, and catalyst concentration) which provide the highest reaction conversion, selectivity, and biodiesel yield. The BBD experimental design is shown in **Table 4**.

Table 4. Experimental Design of BBD.

Run	Temp (°C)	Ratio	Catalyst Conc (%)	Conversion %			Selectivity %			Yield %		
				Exp	Predict	Error	Exp	Predict	Error	Exp	Predict	Error
1	30	6	2.5	49.55	48.51	2.10	14.72	13.69	7.02	7.29	6.59	9.62
2	30	9	5	37.95	38.21	0.68	15.52	16.28	4.87	5.89	5.88	0.17
3	50	6	2.5	50.35	50.21	0.28	13.93	12.07	13.36	7.01	6.21	11.35
4	40	6	5	49.80	44.49	10.66	15.13	13.00	14.09	7.54	6.16	18.33
5	40	3	7.5	38.15	39.55	3.68	11.62	10.65	8.36	4.43	4.57	3.13
6	40	3	2.5	39.95	43.28	8.33	7.36	10.41	41.43	2.94	3.58	21.64
7	50	9	5	45.70	46.26	1.23	14.61	14.66	0.33	6.68	7.30	9.28
8	30	6	7.5	45.40	44.78	1.35	12.71	13.93	9.57	5.77	6.10	5.75
9	40	6	5	38.95	44.49	14.23	10.59	13.00	22.73	4.12	6.16	49.46
10	40	9	7.5	42.05	43.38	3.16	14.64	15.59	6.46	6.16	5.61	8.99
11	30	3	5	43.25	40.73	5.82	13.54	11.34	16.27	5.86	5.16	11.99
12	40	9	2.5	49.25	47.10	4.36	15.49	15.35	0.93	7.63	7.57	0.74
13	40	6	5	41.70	44.49	6.70	13.43	13.00	3.22	5.60	6.16	9.96
14	50	6	7.5	48.60	46.48	4.35	13.49	12.31	8.76	6.56	5.73	12.71
15	50	3	5	38.30	36.08	5.78	7.99	9.72	21.65	3.06	2.99	2.37
16	40	6	5	42.55	44.49	4.56	13.46	13.00	3.44	5.73	6.16	7.47
17	40	6	5	45.55	44.49	2.32	12.73	13.00	2.10	5.80	6.16	6.17

3.2.1. Reaction Conversion

According to Jookjantra & Wongwuttanasatian^[28], there are four polynomial models that can be applied to predict experimental data response variables, namely, linear, interactive (2FI), quadratic and cubic models. To select the most suitable model for the conversion and selectivity of biodiesel produced from nyamplung seed oil, several parameters are needed such as sequential p-value, lack of fit p-value, adjusted R², predicted R² and Adiq precision. In this research, to optimize conversion, a quadratic model is used which can be presented in **Table 5**.

Based on the result presented in **Table 5**, quadratic model showed the p-value of 0.1673, in which the value didn't fulfil the requirement of p-value < 0.05. Thus, this model was not significant. To overwhelm this difficulty, modification on the model was carried out to obtain the significant model^[29]. ANOVA with the modified cubic model called reduced cubic model is depicted in **Table 6**.

Table 5. ANOVA Quadratic Model for Reaction Conversion.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	232.36	9	25.82	2.12	0.1673	significant
A-Suhu	5.78	1	5.78	0.4743	0.5132	
B-Rasio	29.26	1	29.26	2.4	0.1652	
C-Katalis	27.75	1	27.75	2.28	0.175	
AB	40.32	1	40.32	3.31	0.1117	
AC	1.44	1	1.44	0.1182	0.7411	
BC	7.29	1	7.29	0.5982	0.4646	
A ²	14.53	1	14.53	1.19	0.311	
B ²	76.68	1	76.68	6.29	0.0405	
C ²	35.59	1	35.59	2.92	0.1312	
Residual	85.3	7	12.19			
Lack of Fit	16.78	3	5.59	0.3266	0.8076	not significant
Pure Error	68.52	4	17.13			
Cor Total	317.66	16				

Table 6. ANOVA For Reduced Qubic Model for Reacton Conversion.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	209.11	6	34.85	3.21	0.0503	significant
A-Suhu	5.78	1	5.78	0.5324	0.4823	
B-Rasio	29.26	1	29.26	2.70	0.1317	
C-Katalis	27.75	1	27.75	2.56	0.1409	
AB	40.32	1	40.32	3.71	0.0828	
B ²	73.41	1	73.41	6.76	0.0265	
C ²	38.13	1	38.13	3.51	0.0904	
Residual	108.56	10	10.86			
Lack of Fit	40.04	6	6.67	0.3896	0.8548	not significant
Pure Error	68.52	4	17.13			
Adeq Prec	6.68					
Cor Total	317.66	16				

Table 6 exhibits the p-value for the model was 0.0503, in which this value did not meet the p-value requirements. However, in B^2 , the value met the p-value requirements. In this cubic model, the lack of fit obtained is not significant. Insignificant information obtained on lack of fit may be used for prediction. The adeq precision value is a measure of the signal to noise ratio and the expected ratio is > 4 [30]. In this model the Adeq precision value was 6.68, showing that the model was satisfactory for use. Thus, it can be deduced that the cubic model can be used to describe the reaction conversion response. The empirical relationship shown between the cubic model and the interactions between variables will be converted into an order polynomial form. The equation for optimizing conversion can be seen in Equation (1).

$$\text{Conversion (\%)} = 44.49 + 0.85 A + 1.91 B - 1.86 C + 3.18 AB - 4.17 B^2 + 3.01 C^2 \quad (1)$$

where,

A: temperature ($^{\circ}\text{C}$)

B: molar ratio of ethyl acetate to il (mol)

C: catalyst concentration (%)

3.2.2. Selectivity

The ANOVA regression model for selectivity uses the reduced quadratic model which is presented in **Table 7**. Based on the data analysis using ANOVA, it was revealed that the model had a p-value of 0.0313, which met the requirements for a p-value < 0.05 . Among the variables, only the molar ratio parameter provided a value that satisfy the p-value requirements. The adeq precision value is a measure of the signal to noise ratio, in which the expected ratio is > 4 [31]. In this model, the Adeq precision value obtained was 7.38, indicating that this model was appropriate for implementation for use. Lack of fit attained was not significant, meaning that it can be applied for predictions.

Table 7. ANOVA for Linear Model (Selectivity).

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	54.11	3	18.04	6.20	0.0076	significant
A-Suhu	5.23	1	5.23	1.80	0.2027	
B-Rasio	48.76	1	48.76	16.77	0.0013	
C-Katalis	0.1152	1	0.1152	0.0396	0.8453	
Residual	37.79	13	2.91			
Lack of Fit	27.00	9	3.00	1.11	0.4967	not significant
Pure Error	10.79	4	2.70			
Adeq Prec	7.92					
Cor Total	91.90	16				

The empirical relationship shown between the cubic model and the interactions between variables will be converted into a 2nd order polynomial form. The equation for optimizing selectivity can be seen in Equation (2).

$$\text{Selectivity (\%)} = 13 - 0.81 A + 2.47 B + 0.12 C \quad (2)$$

Where,

A: temperature ($^{\circ}\text{C}$)

B: molar ratio of ethyl acetate to oil (mol)

C: catalyst concentration (%)

3.2.3. Biodiesel Yield

The ANOVA regression model for yield applied a reduced quadratic model which is presented in **Table 8**. ANOVA analysis on the data presented in Table shows that the model had a P-value of 0.0318, which was in line with the requirements for a p-value < 0.05. The adeq precision value is a measure of the signal to noise ratio and the expected ratio is > 4^[32]. In this model, the Adeq precision value achieved was 7.07, revealing that this model was proper for use. Lack of fit obtained was not significant, stipulating that it may be used for predictions.

Table 8. ANOVA For Reduced Cubic Model (Yield).

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	21.74	6	3.62	3.54	0.0381	significant
A-Suhu	0.2812	1	0.2812	0.2749	0.6115	
B-Rasio	12.68	1	12.68	12.39	0.0055	
C-Katalis	0.4753	1	0.4753	0.4645	0.5110	
AB	3.22	1	3.22	3.15	0.1064	
BC	2.19	1	2.19	2.14	0.1741	
B ²	2.89	1	2.89	2.83	0.1236	
Residual	10.23	10	1.02			
Lack of Fit	4.35	6	0.7243	0.4922	0.7912	not significant
Pure Error	5.89	4	1.47			
Adeq Prec	7.07					
Cor Total	31.97	16				

The empirical relationship shown between the cubic model and the interactions between variables will be converted into a 2nd order polynomial form. The equation for optimizing yield can be seen in Equation (3)

$$\text{Yield (\%)} = 6.16 - 0.19 A + 1,26 B - 0.24 C + 0.90 AB - 0.74 BC - 0.82 B^2 \quad (3)$$

where

A: temperature (°C)

B: molar ratio (mol)

C: catalyst concentration (%)

3.3. Process Parameters Based on RSM Analysis

The influence of process variables such as temperature, molar ratio, and catalyst concentration on reaction conversion, selectivity, and biodiesel yield was evaluated using Response Surface Methodology. Based on the model obtained, an analysis of the effects and interactions of process variables on response was carried out using 3D RSM and numerical optimization.

3.3.1. Reaction Conversion

The interaction between independent variables on reaction conversion is shown in **Figure 2**. **Figure 2(a)** describes the interaction between reaction temperature and molar ratio which provides quite significant changes in increasing conversion. It can be seen that the reaction conversion began to increase at a temperature of 50°C and a molar ratio of ethyl acetate to oil of 9. This finding was in line with the analysis carried out by Zainal et al.^[11] which stated that the optimum point at a temperature of 45°C and a molar ratio of 1:9 for the enzymatic ultrasonic-interesterification. **Figure 2(b)** demonstrates that the increasing of the catalyst concentration did not provide a significant change in conversion of the reaction. **Figure 2(c)** exhibits that the

greater the molar ratio used, the resulting conversion will enhance. Surprisingly, Sendzikiene and Makareviciene^[33] suggested the molar ratio of 18: 1 to 80: 1.

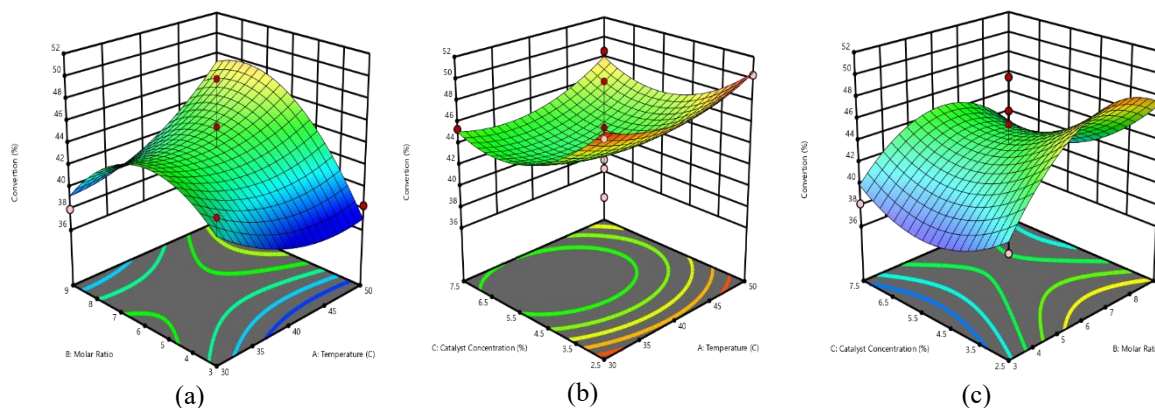


Figure 2. 3D Graph of Process Parameter on Conversion.

The Derringer method is used to optimize the response which is characterized by the desirability function. Desirability shows how close the optimization results are to the optimal point. The desirability function (DF) is built on the transformation of all responses obtained from different scales into scale-free values^[15]. The DF value ranges from 0 to 1. A value of 0 means the factor gave an undesirable response. On the other hand, a desirability value close to 1 is the expected value because it indicates optimal performance of the factor being evaluated. However, analysis using RSM does not only aim at a desirability value equal to 1, but rather to determine conditions that are in line with expectations. Based on the results of the RSM analysis, it was found that the highest reaction conversion was 47.4% at a temperature of 50°C, a molar ratio of 8.99, and a catalyst concentration of 7.49%. This condition was close to the optimum point where the desirability value obtained was 0.888 as shown in Figure 3. The result obtained was higher than the work of Winayanuwattikun et al.^[34], which generate biodiesel product of 43.8%, 25%, and 41.3% for the process carried out at 55°C and catalyst concentration of 20%, and using the feedstocks of physic nut oil, papaya oil, and rambutan oil, respectively. The optimum temperature of 50°C was comparable with the work of Li et al.^[35]. However, they applied higher enzyme catalyst concentration of 15.9%.

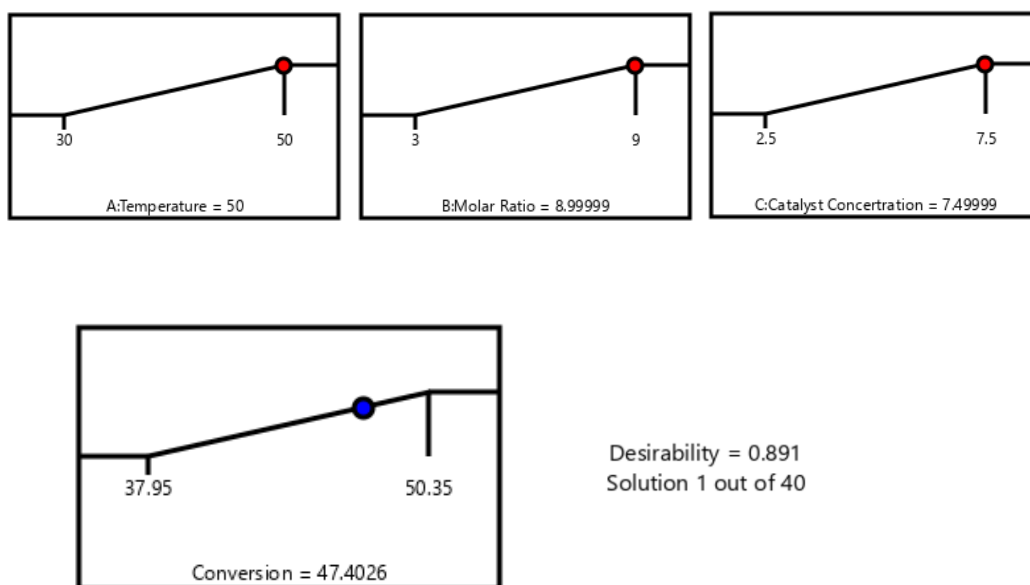


Figure 3. Optimization of Conversion.

3.3.2. Selectivity

Based on **Figure 4**, it can be observed the relationship between reaction temperature, molar ratio of ethyl acetate to oil, and catalyst concentration on reaction selectivity. The optimum selectivity achieved was 14.78%. **Figure 4(a)** shows the interaction of temperature and ratio, presenting that the increasing temperature and ratio provided a significant influence on the increasing of biodiesel selectivity. The relationship between temperature and catalyst is exhibited in **Figure 4(b)**, showing that temperature variations did not have a significant effect on increasing selectivity. **Figure 4(c)** describes the interaction of catalyst concentration and molar ratio of ethyl acetate to oil on the selectivity. Increasing the molar ratio gave a significant influence on selectivity. It was indicated that the selectivity increased by the rising of molar ratio from 6:1 to 9:1 with a catalyst concentration of 2.5%. Interesterification reaction of oil and ethyl acetate is a reversible reaction. Due to the thermodynamic principle, the excessive methyl acetate of ethyl acetate in the interesterification reaction will alter the equilibrium towards biodiesel product formation^[36]. Youssef et al.^[37] depicted that the increase of reactant molar ratio in the interesterification reaction can promote the mass transfer of the reactants. Therefore, it increases reaction conversion, selectivity, and yield of biodiesel.

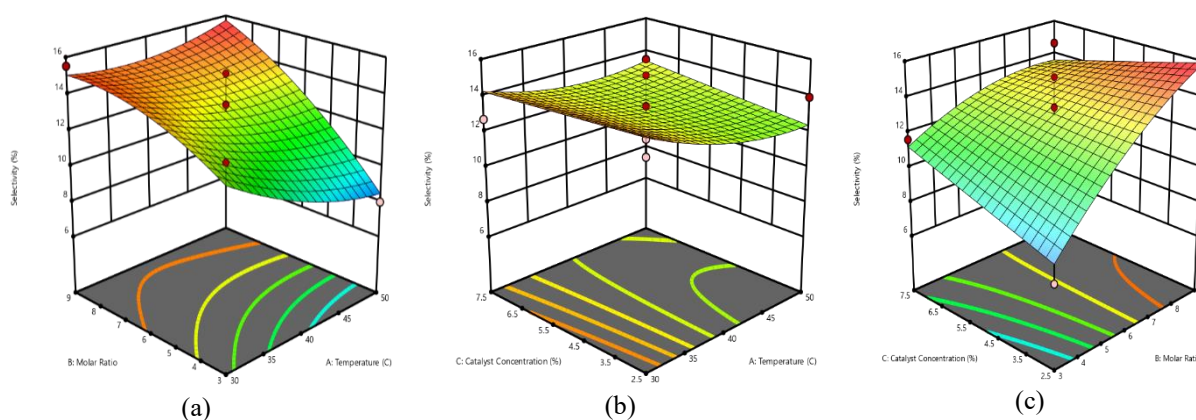


Figure 4. 3D Graph of Process Parameter on Selectivity.

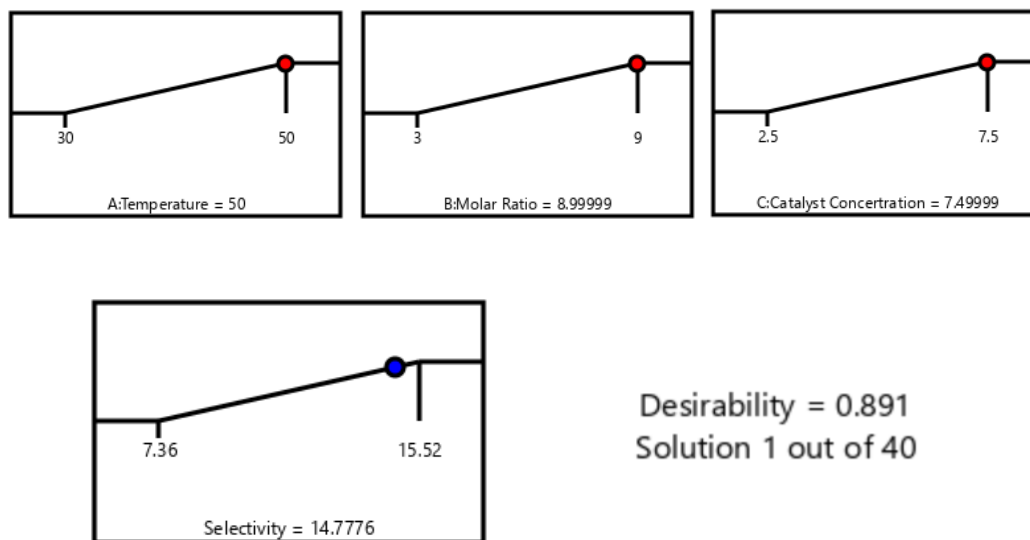


Figure 5. Optimization of Selectivity.

Based on the results of the RSM analysis, the selectivity was 14.78% at a temperature of 50°C, a molar ratio of 8.99, and a catalyst concentration of 7.49%. This condition is close to the optimum point where the desirability value obtained is 0.891 as shown in **Figure 5**.

3.3.3. Biodiesel Yield

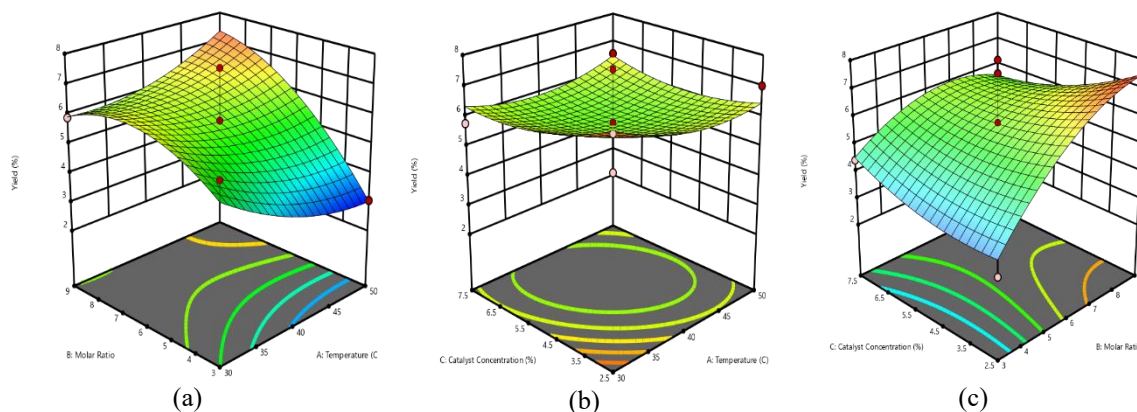


Figure 6. 3D Graph of Process Parameter on Yield.

Figure 6 presents the relationship between reaction temperature, molar ratio of ethyl acetate to oil, and catalyst concentration on biodiesel yield. Figure 6(a) shows the interaction of reaction temperature and molar ratio. It was demonstrated that the rising of the temperature and molar ratio resulted in a significant influence to improve biodiesel yield. On the other hand, Figure 6(b) points that increasing of the catalyst concentration and temperature did not generate significant changes on the biodiesel yield. Figure 6(c) expressed the interaction of catalyst and molar ratio of ethyl acetate to oil. Increasing of the molar ratio designated a significant effect on yield. Intensification on biodiesel yield occurred for the molar ration in the range of 6:1 to 9:1 with a catalyst concentration of 2.5%. Based on RSM optimization, it was discovered that the optimum yield was 6.32% achieved at a temperature, molar ratio of ethyl acetate to oil, and catalyst concentration of 50°C, 8.99, and 7.49%, respectively. This condition is close to the optimum point where the desirability value obtained is 0.891 as shown in Figure 7. Biodiesel yield resulted in this work was lower than that reported by Nor et al.^[36], which was conducted using ultrasonic-assisted interesterification reactor. This is due to the fact that enzymatic reaction is slow. However, ultrasonic irradiation can create cavitation which enhance the mixing and mass transfer between reactants and thus increasing biodiesel yield^[37]. Besides, ultrasonic reaction at low frequency can shorten the reaction time^[38]. Therefore, biodiesel yield in this work can be improved by applying ultrasonic-assisted reactor. In the absence of ultrasonic irradiation, enzymatic reaction was performed with long reaction time, such as 24 hours^[39] or 96 hours^[40], which were extremely longer than the reaction time applied in this work (8 hours). Since the yield of biodiesel in this work is not high enough, the physicochemical properties of biodiesel were not evaluated.

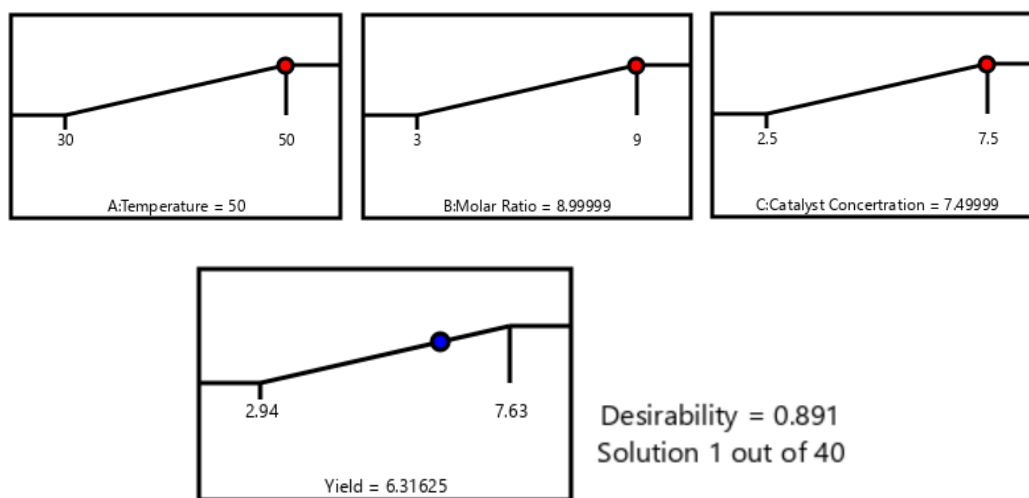


Figure 7. Optimization of Yield.

4. Conclusion

Based on the experimental data, it can be concluded that temperature and molar ratio of reactants had significant effects on the reaction conversion, selectivity and biodiesel yield of interesterification reaction of nyamplung seed oil with ethyl acetate using an immobilized lipase catalyst. The maximum reaction conversion was 49.25% with the yield of 7.63% and selectivity of 15.49%, obtained at a temperature, molar ratio of reactants, and immobilized lipase catalyst concentration of 40°C, 1:9 and 2.5%, respectively. Based on RSM analysis and optimization using BBD, it was found that temperature, molar ratio of the reactant, and catalyst concentration showed significant effects on the reaction conversion, selectivity and biodiesel yield of interesterification reaction of nyamplung seed oil with ethyl acetate employing immobilized lipase catalyst. The RSM analysis results showed that the optimum reaction conversion were 47.40% with selectivity of 14.78% and biodiesel yield of 6.32% at a temperature, molar ratio of reactants, and immobilized lipase catalyst concentration of 50°C, 1:8.99, and 7.49%, consecutively.

Author contributions

Conceptualization, Ratna Dewi Kusumaningtyas; methodology, Ratna Dewi Kusumaningtyas, Nuni Widiarti; software, Ratna Dewi Kusumaningtyas, Nuni Widiarti; validation, Dwi Widjanarko, Hasan Maksum, Wawan Purwanto, Rifdaman, Sucihatiningsih Dian Wisika Prajanti; formal analysis, Ratna Dewi Kusumaningtyas, Nuni Widiarti; investigation, Dya Ayu Septiyan, Noviana Dias Pratiwi; resources, Ratna Dewi Kusumaningtyas; data curation, Ratna Dewi Kusumaningtyas, Nuni Widiarti; writing—original draft preparation, Ratna Dewi Kusumaningtyas; writing—review and editing, Ratna Dewi Kusumaningtyas and Dwi Widjanarko; visualization, Ratna Dewi Kusumaningtyas, Dya Ayu Septiyan, Noviana Dias Pratiwi, Rizky Ichwan; supervision, Ratna Dewi Kusumaningtyas, Nuni Widiarti; project administration, Dya Ayu Septiyan, Noviana Dias Pratiwi; funding acquisition, Ratna Dewi Kusumaningtyas. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Wang H, Chen J, Pei Z, Fang Z, Yang S, Li H. Bio-based deep eutectic solvent of enhanced lignin solubility for wheat straw fractionation and full-component utilization. *Industrial Crops and Products* 2025 Jan 1; 223: 120054.
2. Yuan J, Li J, Huang J, Wu H, Tan J, Li H. Pyro-assisted deep eutectic solvents pretreatment of lignocellulosic biomass: A review. *Industrial Crops and Products* 2024 Dec 15; 222: 119641.
3. Chaudhary A, Rathour RK, Solanki P, Kakkar PM, Pathania S, Walia A, Baadhe RR, Bhatia RK. Recent technological advancements in biomass conversion to biofuels and bioenergy for circular economy roadmap. *Renewable Energy* 2025 May 1; 244: 122714.
4. Tan J, Yu D, Yuan J, Wu H, Luo H, Zhang H, Li X, Li H, Yang S. Efficient delignification of wheat straw for microbial lipid production enabled by a novel ternary deep eutectic solvent containing ethylene glycol. *Fuel* 2023 Sep 1; 347: 128485.

5. Ruatpuia JV, Halder G, Vanlalchhandama M, et al. *Jatropha curcas* oil a potential feedstock for biodiesel production: A critical review. *Fuel* 2024; 370: 131829.
6. Kusumaningtyas RD, Kusuma ADH, Budiono YWP, et al. Biodiesel production from used cooking oil using integrated double column reactive distillation: simulation study. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 2022; 94(1): 152.
7. Kusumaningtyasa RD, Handayani PA, Rochmadi, et al. Tin (II) Chloride Catalyzed Esterification of High FFA *Jatropha* Oil: Experimental and Kinetics Study. Semarang: *International Journal of Renewable Energy Development* 2014; 3 (2): 75.
8. Astuti E, Mufrodi Z, Linarti U, et al. Performance Analysis of Biodiesel Derived from Alkali Catalyzed Transesterification of Waste Cooking Oil. *International Journal of Renewable Energy Research (IJRER)* 2023; 13(3): 1145.
9. Mufrodi Z, Budiman A, Purwono S. Operation conditions in synthesize of bioaditive from glycerol as by-product biodiesel: a review. *Energy Procedia* 2018; 145: 434.
10. Shohel MA, Islam MA, Siam MT. Evaluation of activation, performance and reusability between eggshell-derived heterogenous and homogenous catalysts for neem oil biodiesel. *Chemical Engineering Journal Advances* 2026 Jan 15:101045.
11. Zainal MZA, Veny H, Hamzah F, et al. Enzymatic Interesterification of Crude Palm Oil with Methyl acetate: Effect of Pre-treatment, Enzyme's Dosage and Stability. *Bulletin of Chemical Reaction Engineering & Catalysis* 2023; 18(2): 294.
12. Badoei-dalfard A, Malekabadi S, Karami Z, et al. Magnetic cross-linked enzyme aggregates of Km12 lipase: A stable nanobiocatalyst for biodiesel synthesis from waste cooking oil. *Renewable energy* 2019; 141: 874.
13. Khan MR Immobilized enzymes: a comprehensive review. *Bulletin of the National Research Centre* 2021; 45(1) : 207.
14. Yousefi M, Marciello M, Guisan JM, et al. Fine modulation of the catalytic properties of *Rhizomucor miehei* lipase driven by different immobilization strategies for the selective hydrolysis of fish oil. *Molecules* 2020; 25(3): 545.
15. Kusumaningtyas RD, Prasetiawan H, Anggraeni ND, et al. Conversion of Free Fatty Acid in *Calophyllum inophyllum* Oil to Fatty Acid Ester as Precursor of Bio-Based Epoxy Plasticizer via SnCl₂-Catalyzed Esterification. *Polymers* 2023; 15(1): 123.
16. Nabizadeh R, García IL, Sadjadi S, et al., Biodiesel production from supernatant waste cooking oil by a simple one-step technique: calorific value optimization using response surface methodology (RSM) based on D-optimal design. *Journal of Material Cycles and Waste Management* 2023; 25(6): 3567.
17. Xu H, Yin B, Liu S, et al. Performance optimization of diesel engine fueled with diesel–*jatropha curcas* biodiesel blend using response surface methodology. *Journal of Mechanical Science and Technology* 2017; 31(8): 4051.
18. Nouadjep NS, Nso E, Kana EG, et al. Simplex lattice mixture design application for biodiesel production: Formulation and characterization of hybrid oil as feedstock. *Fuel* 2019; 252: 135.
19. Rossi DM, de Souza EA, Flôres SH, et al. Conversion of residual glycerol from biodiesel synthesis into 1, 3-propanediol by a new strain of *Klebsiella pneumoniae*. *Renewable Energy* 2013; 5: 404.
20. Yesilyurt MK, Cesur C. A statistical optimization attempt by applying the Taguchi technique for the optimum transesterification process parameters in the production of biodiesel from *Papaver somniferum* L. seed oil. *Fuel* 2022; 329: 125406.
21. Myers RH, Montgomery DC, Anderson-Cook CM, *Response surface methodology: process and product optimization using designed experiments*. In Third Edition. John Wiley & Sons; 2009.
22. Nidzam MS, Hossain MS, Ismail N, et al. Influence of the degumming process parameters on the formation of glyceryl esters and 3-MCPDE in refined palm oil: optimization and palm oil quality analyses. *Foods* 2022; 11(01): 124.
23. Chen X, Sun S. Color reversion of refined vegetable oils: A review. *Molecules* 2023; 28(13): 5177.
24. Adekunle AS, Oyekunle JAO, Obisesan OR, et al. Effects of degumming on biodiesel properties of some non-conventional seedoils. *Energy Reports* 2016; 2: 188
25. Chew SC, Tan CP, Nyam KL. Optimization of degumming parameters in chemical refining process to reduce phosphorus contents in kenaf seed oil. *Separation and Purification Technology* 2017 Nov 29; 188: 379-85.
26. Miesiac I, Rogalinski A, Jozwiak P, Transesterification of triglycerides with ethyl acetate. *Fuel* 2013; 105, 169 (2013)
27. Takase M. Biodiesel yield and conversion percentage from waste frying oil using fish shell at elmina as a heterogeneous catalyst and the kinetics of the reaction. *International Journal of Chemical Engineering* 2022; 2022(1): 8718638.
28. Jookjantra K, Wongwuttanasatian T, Optimisation of biodiesel production from refined palm oil with heterogeneous CaO catalyst using pulse ultrasonic waves under a vacuum condition. *Energy Conversion and Management* 2017; 154, 1.
29. Handayani PA, Abdullah A, Hadiyanto H, Response surface optimization of biodiesel production from *Nyamplung* (*Calophyllum inophyllum*) oil enhanced by microwave and ionic liquid+ NaOH catalyst. *Periodica Polytechnica Chemical Engineering* 2019; 63(3): 406.

30. Yang L, Wang L, Gao J, et al. Optimization of process parameters for preparing metallic matrix diamond tool bits by microwave pressureless sintering using response surface methodology. *Materials* 2018; 11(11): 2185.
31. Srikanth HV, Venkatesh J, Godiganur S, Box-Behnken response surface methodology for optimization of process parameters for dairy washed milk scum biodiesel production. *Biofuels* 2021; 12(1):113.
32. Yin H, Fan S, Peng K, et al. Process optimization for improving anti-oxidation performance of silver-coated copper powders by response surface methodology and artificial neural network. *Materials & Design* 2025; 253:113855.
33. Sendzikiene E, Makareviciene V, Synthesis of biodiesel by transesterification of triglycerides with Methyl formate. *Applied Sciences* 2022; 12(19): 9912.
34. Winayanuwattikun P, Kaewpiboon C, Piriyananon K, et al. Potential plant oil feedstock for lipase-catalyzed biodiesel production in Thailand. *Biomass and bioenergy* 2008; 32(12):1279.
35. Li Q, Chen Y, Bai S, et al. Immobilized lipase in bio-based metal-organic frameworks constructed by biomimetic mineralization: A sustainable biocatalyst for biodiesel synthesis. *Colloids and surfaces B: Biointerfaces* 2020; 1:18
36. Nor NFSM., Veny H, Hamzah F, et al. Enzymatic Transesterification Using Different Immobilized Lipases and its Biodiesel Effect on Gas Emission. *Bulletin of Chemical Reaction Engineering & Catalysis* 2024; 19(2): 265.
37. Youssef O, Khaled E, Aboelazayem O, et al. Glycerol-Free Biodiesel via Catalytic Transesterification: A Pathway to a NetZero Biodiesel Industry. *Sustainability* 2024; 6(12): 4994.
38. Siaw WC, Tan LS, Kiew PL, et al. Synthesis of Biodiesel from Palm Oil Using a Hybrid Mechanical Stirring and High Frequency Ultrasonic-Assisted Enzyme-Catalysed Process. *Chemical Engineering Transactions* 2023; 106: 1057.
39. Bhangu SK, Gupta S, Ashokkumar M. Ultrasonic enhancement of lipase-catalysed transesterification for biodiesel synthesis. *Ultrasonics Sonochemistry* 2016; 34: 305.
40. Zhong L, Jiao X, Hu H, et al. Activated magnetic lipase-inorganic hybrid nanoflowers: A highly active and recyclable nanobiocatalyst for biodiesel production. *Renewable Energy* 2021; 171: 825.
41. Babaki M, Yousefi M, Habibi Z, et al. Preparation of highly reusable biocatalysts by immobilization of lipases on epoxy-functionalized silica for production of biodiesel from canola oil. *Biochemical Engineering Journal* 2015; 101: 23.