EVALUATION OF THE KINETICS OF A LIPASE CATALYSED BIODIESEL PRODUCTION FROM A MIXTURE OF WASTE COOKING OIL AND SOYBEAN OIL

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ABSTRACT
Commercial lipase Novozym 435 was used to catalyse the transesterification of a mixture of waste cooking oil and soybean oil with methanol. A central composite experimental design was performed to analyze the effects of lipase dosage, waste cooking oil content in the mixture, and reaction time on the methyl ester yield. Lipase dosage and waste cooking oil content were the most significant variables after statistical analysis. The highest yield, 98.04%, was obtained using 12.5% of lipase, a mixture containing 50% of waste cooking oil after 10 h of reaction. The lipase was reutilized and showed a good operational stability since the yield was reduced by 6% even after five batches. Two kinetic models, Power Law and Eley-Rideal, were used to model the production process and correlate the experimental results.

KEYWORDS
experimental design; lipase; Eley-Rideal mechanism; oil mixture; methanol

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1. INTRODUCTION

Nowadays, most of the energy produced in the world comes from fossil fuels. However, the increase of environmental damage caused by pollutants emissions due to the use of fossil fuel leads to an intensification of the search for renewable sources of energy. An alternative to the use of fossil fuels, biodiesel is a biofuel produced from vegetable oils and animal fats (Karmakar et al., 2010) by the transesterification of triglycerides with a short chain alcohol in the presence of a catalyst (Robles-Medina et al., 2009).

The basic homogeneous catalyst is the most used in industrial scale. Since the use of this catalyst has some drawbacks, such as demand for high energy, need for removing organic salts, and treating alkaline wastewater, the development of biocatalysts has become a promising alternative for biofuel production. This biochemical approach is considered a less aggressive alternative for the environment, and its products present a higher level of purity (Tan et al., 2010; Atadashi et al., 2013).

The uses of lipases have received great attention because of their features such as mild operation conditions, high selectivity, and high specificity. Moreover, raw materials with high content of free fatty acids do not interfere on biodiesel conversion, and their enzymes can be recovered easily and reused in many cycles (Christopher et al., 2014). Many immobilized lipases are commercially available, among those Novozym 435 (immobilized lipase B of Candida antarctica) stands out due to its fast reaction rate and high availability worldwide (Avhad & Marchetti, 2015). Nevertheless, there are still some problems related to the use of lipases, such as the inhibition caused by alcohol, the long reaction time, and its high cost (Bajaj et al., 2010).

Another factor that influences the economic feasibility of biodiesel production is the cost of raw materials, since the cost of farming and extracting oil accounts for, nearly, one third of the total cost of production (de Araújo et al., 2013; Mahmudul et al., 2017). The most common raw materials used in the production of biodiesel are refined edible oils, but some problems associated with their uses are the high cost of production and the demand competition with the food industry. Hence, the use of animal fat, non-edible oils, and waste frying oil have gained more attention as sources of triglycerides.

However, characteristics such as high viscosity, lower availability, and use in industries as a source of biolubricants make theses alternative raw materials non self-sufficient for biodiesel production (Ashrafual et al., 2014). Therefore, the mixture of edible and non-edible oils can be a way to overcome these problems, and to diversify the origin of raw materials used (Azócar et al., 2010; de Almeida et al., 2015; Gupta et al., 2016).

Three of the largest biodiesel producers in the world, the United States, Brazil, and Argentina, use soybean oil as the main raw material (Mahmudul et al., 2017). This is due to easy adaptability of cultivation and the fact that these countries already cultivate soy on a large scale to produce protein meal (de Oliveira & Coelho, 2017).

Nevertheless, the use of waste frying oil as raw material for biodiesel is not as widespread in an industrial scale, but it can be considered a promising alternative. The cost of biodiesel production can be reduced by two or three times when refined oils are replaced by residual cooking oil (Lam et al., 2010). Although this raw material contains high fatty acids and water amounts, which results in soap formation with chemical catalysts, many studies have proved that biodiesel production from waste frying oil is possible because the steps of transesterification and esterification occur simultaneously, mainly when a lipase is used (de Araújo et al., 2013; Talebian-Kiakalaieh et al., 2013).

Several studies report the process of enzymatic transesterification of vegetable oils, but only 3 studies analyze biodiesel production using oil mixtures and lipases (Pizarro & Park, 2003; Azócar et al., 2010; Véras et al., 2011).

Therefore, our goal was to evaluate the main operating conditions that affect the enzymatic transesterification reaction using a mixture of soybean oil and waste cooking oil as raw materials. We also analyzed the reusability of the lipase and studied the kinetics of the reaction.
2. MATERIALS AND METHODS

2.1 Materials

Novozym 435 (lipase B from Candida Antarctica immobilized on macroporous polyacrylic resin beads) was donated by Novozymes Latin America Ltd. (Rio de Janeiro, Brazil).

Soybean oil was purchased from a local market. Waste cooking oil (WCO) was donated by local restaurants (Rio de Janeiro, Brazil), and it was filtrated to remove solid particles. Table 1 shows the physicochemical properties of the oils. The analyses were performed according to ASTM D664, ASTM D4052, and ASTM D445 for acidiy index, density, and viscosity, respectively.

Methanol (99.8%), ethanol (99.5%), and hexane P.A. were purchased from Isofar Indústria e Comércio de Produtos Ltda (Rio de Janeiro, Brazil). Ethyl ether P.A., sodium hidroxide P.A., anhydrous sodium sulfate, and phenolphthalein were purchased from Vetec Química Fina Ltda (Rio de Janeiro, Brazil). Boron trifluoride complex was purchased from Sigma-Aldrich (Rio de Janeiro, Brazil).

2.2 Experimental design

A two-level-three factor central composite design was performed with six axial points, and a central point done in triplicate, totaling 17 runs. The experiments were randomized to minimize errors. Lipase dosage ($X_1$), waste cooking oil content ($X_2$), and time reaction ($X_3$) were chosen as independent variables to optimize the transesterification reaction. Coded and uncoded (actual) levels of the independent variables are presented in Table 2. Methyl ester ($Y$) was the result (response variable) of the experimental design.

The experimental data was fitted to a second-order polynomial model, Eq. (1), which includes all interaction terms.

$$
Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_i^2 X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j
$$

with $Y$ as the dependent variable, $\beta_0$ the constant coefficient, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ as coefficients for linear, quadratic, and interaction effect, and $X_i$ and $X_j$ as independent variables.

The data were analyzed and interpreted using F-test, analysis of variance (ANOVA), regression analysis, and response surface plot.

2.3 Experimental methodology

All transesterification reactions were carried out in a screw cap test tube with 2 g of oil mixture, and a methanol to oil ratio of 3:1. The reaction media was incubated at 40 °C using a water bath, and maintained under magnetic stirring at 350 rpm. The content of waste cooking oil in the mixture, the lipase content, and time reaction were fixed according to the experimental design. At the end of the reaction, the sample was centrifuged for 10 min at 2500 rpm. An aliquot was taken from the upper phase, and it was dissolved in hexane before performing the chromatographic analysis.

Table 1. Physicochemical properties of soybean oil and waste cooking oil.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Soybean oil</th>
<th>Waste cooking oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity index (mg KOH/g)</td>
<td>0.309</td>
<td>18.809</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>0.907</td>
<td>0.909</td>
</tr>
<tr>
<td>Viscosity at 42 ºC (mm²/s)</td>
<td>34.598</td>
<td>40.915</td>
</tr>
</tbody>
</table>

Table 2. Independent variables and their levels.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Name</th>
<th>Coded Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1.68</td>
</tr>
<tr>
<td>X₁</td>
<td>Lipase (%)</td>
<td>5</td>
</tr>
<tr>
<td>X₂</td>
<td>WCO (%)</td>
<td>0</td>
</tr>
<tr>
<td>X₃</td>
<td>Reaction time (h:min)</td>
<td>04:00</td>
</tr>
</tbody>
</table>
2.4 Analytical method

The fatty acid compositions of each oil were determined from the derivation process, following the EC 2-66 AOCS method to avoid clogging of the column, and they were analyzed using a Hewlett-Packard 6890 gas chromatography equipped with a DB-17HT column (30 m x 0.25 mm x 0.15 µm) coupled to a mass spectrometer (MS).

The methyl ester composition was also quantified using a Hewlett-Packard 6890 gas chromatography equipped with a DB-17HT column, but coupled to a flame ionizing detector (FID). The column temperature was initiated at 110 ºC, heated to 365 ºC at a rate of 5 ºC/min. Injector and detector temperatures were set at 250 ºC. The split ratio was 1:50, and hydrogen was used as a carrier gas at a flow rate of 1 mL/min. The amount of sample injected was 2 µL, and the total analysis time was 35 min. The methyl ester yield was defined as the sum of the percentage of all methyl esters identified and quantified by the chromatographic analysis.

2.5 Enzyme reuse

The operational stability of an immobilized lipase is of great importance to the economic viability of any biocatalytic process. Therefore, after completing all the essays from the experimental design, lipase was tested for its reusability. After every production process, the biocatalyst was washed three times with n-hexane, and then, filtered under vacuum using a sintered glass funnel. Several works report the use of n-hexane due to its efficiency in removing remaining methanol and glycerol from lipase active sites after biocatalyst separation, improving lipase stability (Rodrigues et al., 2010). The operational conditions used to test the activity of the lipase in more than one cycle were selected from the experiment that gave the highest yield.

2.6 Kinetic modelling

The models normally used to represent the enzymatic transesterification apply the Ping Pong Bi Bi mechanism to describe the reaction (Halim & Kamaruddin, 2008; Chesterfield et al., 2012; Basri et al., 2013; Gofferjé et al., 2014; Azócar et al., 2014). However, the practical application of this model requires the performance of many experiments and analyses, making the process more laborious and less cost-effective. Thus, this work suggests two models to describe this reaction kinetic, one based on the power law and another based on the Eley-Rideal mechanism.

2.6.1 Power-law model

The transesterification reaction is a reversible reaction where one mole of triglyceride (TG) reacts with three moles of methanol (ROH) to produce three moles of methyl ester (FAME) and one mole of glycerol (GL). This work uses only the overall transesterification reaction, Eq. 2, to determine the kinetics, since the concentration of diglycerides and monoglycerides are negligible compared to oil, methanol, glycerol, and methyl esters concentrations as described by Kroumov et al. (2007) and Firdaus et al. (2016).

\[ \text{TG} + 3 \text{ROH} \rightleftharpoons 3 \text{FAME} + \text{GL} \]  

(2)

From the reaction represented in Eq. 2, it is possible to obtain the general equation of reaction rate, Eq. 3, based on the power law.

\[ r = k_i \times [\text{TG}] \times [\text{ROH}]^3 \cdot k_r \times [\text{FAME}]^3 \times [\text{GL}] \]  

(3)

with \( r \) as the reaction rate, and \( k_i \) and \( k_r \) as the kinetic parameters for direct and reverse reactions, respectively.

The reaction rate is related directly to the concentrations of substrates or products as a function of time. Then, the reaction rate equation can be rewritten as a function of variation of triglycerides concentration by time (\( \frac{d[\text{TG}]}{dt} \)), as shown in Eq. 4.

\[ r = -\frac{d[\text{TG}]}{dt} = k_i \times [\text{TG}] \times [\text{ROH}]^3 - k_r \times [\text{FAME}]^3 \times [\text{GL}] \]  

(4)

2.6.2 Eley-Rideal mechanism

According to the Eley-Rideal mechanism, one of the substrates is chemically absorbed on the active site, and reacts with the second substrate (Hagen, 2006). This mechanism is also used to describe heterogeneous chemical catalytic reactions (Ilgen & Akin, 2012; Van De Steene et al., 2012; Al-Sakkari et al., 2017; Risso et al., 2018), but no report has been found on using this model to describe enzymatic transesterification. Published papers report the use of the Eley-Rideal mechanism to describe the transesterification reaction using alcohol as the substrate that is...
adsorbed in the active site, and having the triglyceride react with the complex formed between the active site and alcohol (Al-Sakkari et al., 2017). This description is similar to what happens in the enzymatic transesterification reaction. However, in this case, the triglyceride is the substrate absorbed on the active site, as describe by Al-Zuhair et al. (2007) and Manoel (2014).

First, the triglyceride (TG) is adsorbed by the active site (S) generating the triglyceride-active site complex (TGS). Then, the surface reaction occurs, where the alcohol molecules (ROH) react with the activated complex (TGS) forming the methyl ester molecules (FAME), and the glycerol-active site complex (GLS). Finally, the desorption of glycerol (GL) occurs, restoring the active site (S). This mechanism is shown by the Eq. 5, 6, and 7.

\[
TG + S \rightleftharpoons TGS \tag{5}
\]

\[
TGS + 3 \text{ ROH} \rightleftharpoons \text{GLS} + 3 \text{ FAME} \tag{6}
\]

\[
\text{GLS} \rightleftharpoons \text{GL} + S \tag{7}
\]

Hence, the reaction rates are described in Eq. 8, 9, and 10.

\[
r_{ad} = k_{ad} \times C_{TG} \times C_V - k_{ad}' \times C_{TGS} \tag{8}
\]

\[
r_s = k_s \times C_{TGS} \times C_{ROH} - k_s' \times C_{FAME} \times C_{GL} \tag{9}
\]

\[
r_d = k_d \times C_{GLS} - k_d' \times C_{GL} \times C_V \tag{10}
\]

with \( r_{ad} \), \( r_s \), and \( r_d \) as the rate of adsorption, superficial, and desorption reaction, respectively; \( C_{TG} \) as the triglycerides concentration; \( C_V \) as the active sites concentration; \( C_{TGS} \) as the complex triglyceride-active site concentration; \( C_{ROH} \) as the methanol concentration; \( C_{FAME} \) as the methyl ester concentration; \( C_{GLS} \) as the complex glycerol-active site concentration; \( C_{GL} \) as glycerol concentrations; and \( k_{ad}, k_{ad}', k_s, k_s', k_d, k_d' \) as kinetic parameters of direct and inverse reactions of adsorption, superficial, and desorption, respectively.

The surface reaction is considered the limiting step. Thus, in a manner analogous to that demonstrated by Al-Sakkari et al. (2017), the final form of the rate equation for this suggested Eley-Rideal model is illustrated in Eq. 11.

\[
r_s = \frac{K \times C_{TG} \times C_{ROH} - K'' \times C_{GL} \times C_{FAME}}{1 + K_{ad}' \times C_{TG} + \frac{1}{K_d} \times C_{GL}} \tag{11}
\]

with \( K, K', K_{ad}', K_d' \) as kinetic parameters.

An experiment was performed for each reaction time to avoid any change in control volume. Then, all experiments were carried out maintaining the same temperature conditions, alcohol to oil molar ratio, WCO content, and lipase dosage; varying only the reaction time. The operational conditions used were the ones that gave the highest yield based on the experimental results.

The minimization of the objective function (FO), shown in Eq. 12, and the calculation of determination coefficient (R²) were applied to find the best model to correlate yield and, consequently, to predict new data.

\[
\text{FO} = \sum_{i=1}^{N} \left( Y_{\text{calc}} - Y_{\text{exp}} \right)^2 \tag{12}
\]

with \( Y_{\text{calc}} \) as calculated methyl ester yield, \( Y_{\text{exp}} \) as experimental methyl ester yield, and \( N \) as the number of experiments.

3. RESULTS AND DISCUSSIONS

3.1 Model fitting and ANOVA

The yield results of all the experiments were presented in Table 3. The yield ranged from 89.0 to 98.05%, where the highest yield was obtained in experiment number 14, using 12.5% lipase, an oil mixture containing 50% of waste cooking oil, and 10 h of reaction time. It was also observed that 7 of the total 17 experiments performed resulted in yields greater than 96.5%, which is the minimum ester content established by ANP (National Petroleum, Natural Gas, and Biofuels Agency) for biodiesel.

The experimental data was adjusted to the proposed quadratic model, Eq. (1). An ANOVA analysis at 95% confidence level, showed in Table 4, was conducted to determine whether the quadratic model was significant or not. In this study, the p-value of the model is smaller than 0.001, suggesting that it is suitable for methyl ester yield prediction. In addition, F-test shows that the
calculated $F$ (19.82636) factor was greater than the tabulated one (3.738892), which also indicates that the model could represent the methyl ester yield. The quadratic model, expressed by Eq. (13), describes the calculated methyl ester yield ($Y$) as a function of lipase dosage ($X_1$), waste cooking oil content ($X_2$), and reaction time ($X_3$), and it can be compared to experimental results. The $R^2$ of 0.89824 shows that the model can be significant, and well predict the response variable.

$$
Y = 95.00977 + 1.99832 X_1 - 0.95210 X_2 + 0.79549 X_3 + 0.60560 X_1 X_2 - 0.47198 X_1 X_3 + 0.42480 X_2 X_3 - 0.62941 X_1^2 + 0.62196 X_2^2 + 0.70894 X_3^2
$$

The statistical analysis of variance (ANOVA) at 95% confidence level was also carried out to determine each model term significance, and the results are presented in Table 5. The analysis of p-value was used to check the significance of each coefficient (Babaki et al., 2017; Poppe et al., 2015).

Based on the established confidence level, one can observe that lipase dosage and WCO content in the oil mixture are the significant variables. It is important to notice that the linear lipase dosage factor is the one that affects the yield most significantly, since its $p$-value is the smallest (0.011870). Moreover, one can observe that the lipase dosage has a positive effect on the methyl ester yield. Wang et al. (2015), Azócar et al. (2010), and Halim and Kamaruddin (2008)
observed the same effect of the Novozym 435 on biodiesel yield using waste cooking oil as raw material. This could be explained by the presence of more numbers of active sites in the reaction medium. However, when there is a high content of lipase, enzyme molecules can be aggregated, preventing the substrate to access to active sites (Poppe et al., 2015; Razack & Duraiarasan, 2016).

Table 5. Regression coefficients, standard error, and p-value corresponding to methyl ester yield.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Regression Coefficients</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>95.00977</td>
<td>0.466610</td>
<td>0.000024</td>
</tr>
<tr>
<td>Main Effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_1$: Lipase</td>
<td>1.99832</td>
<td>0.220148</td>
<td>0.011870</td>
</tr>
<tr>
<td>$X_2$: Waste cooking oil</td>
<td>-0.95210</td>
<td>0.219436</td>
<td>0.049217</td>
</tr>
<tr>
<td>$X_3$: Time</td>
<td>0.79549</td>
<td>0.220148</td>
<td>0.068213</td>
</tr>
<tr>
<td>Quadratic Effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>-0.62941</td>
<td>0.244473</td>
<td>0.119403</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>0.62196</td>
<td>0.241271</td>
<td>0.123695</td>
</tr>
<tr>
<td>$X_3^2$</td>
<td>0.70894</td>
<td>0.244473</td>
<td>0.099167</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
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<tr>
<td>$X_1X_2$</td>
<td>0.60560</td>
<td>0.286568</td>
<td>0.168888</td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>-0.47198</td>
<td>0.286568</td>
<td>0.241314</td>
</tr>
<tr>
<td>$X_2X_3$</td>
<td>0.42480</td>
<td>0.286568</td>
<td>0.276490</td>
</tr>
</tbody>
</table>

Figure 1. Response surface plots of methyl ester yield as a function of the independent variables: (a) effect of lipase dosage and waste cooking oil content; (b) effect of lipase dosage and reaction time; (c) effect of waste cooking oil content and time.
An alternative way to express the effect of each parameter as a function of the yield is the response surface plots. According to the regression model given for all results, the response surfaces generated (Figure 1) illustrate the yields as a function of lipase dosage, WCO content, and reaction time, defining the most suitable conditions that maximize the dependent variable.

Figure 1a shows the interaction between lipase dosage and waste cooking oil content. One can observe that by increasing the lipase dosage, the methyl ester yield also increases. The response surface also revealed that the increase in waste cooking oil content in the mixture leads to a reduction of the yield. However, when the lipase dosage is high (14%), the WCO content interferes less in methyl ester yield, maintaining the yield higher than 97%.

The simultaneous dependence of methyl ester yield on the reaction time and lipase dosage is shown in Figure 1b. As shown in Figure 1a, when the lipase dosage is raised, the yield increases. It also shows that reaction time has a small influence on the yield when compared to lipase dosage.

The dependence of yield on WCO content and time reaction is shown in Figure 1c. Mixtures containing more waste cooking oil require a higher reaction time to obtain a reasonable yield.

After analyzing the generated response surfaces, one can conclude that the optimum yield can be obtained with mixtures with lower WCO content, longer reaction time, and higher amount of lipase. However, when addressing the application of this process in the industry, processes are expected to use smaller amounts of biocatalyst, shorter reaction times, and larger amounts of WCO in the mixture. Also, it is important to point out that the minimum yield required for the product to be considered biodiesel is 96.5%. Therefore, this work proposes to further explore different response surfaces that can be generated from the experimental data. Thus, response surfaces relating the yield to lipase dosage and WCO content were generated for different reaction times, as 10, 7, and 4 h, which are presented in Figure 2.

Figure 2. Response surface plots of methyl ester yield as a function of lipase dosage and waste cooking oil content at a specific reaction time (a) 10 h; (b) 7 h; (c) 4 h.
Figure 2a, operating during 10 h of reaction, shows that it is possible to reach a yield higher than 96.5% using more than 8% of Novozyme 435, regardless of the WCO content in the mixture. Focusing on the 7 h reaction (Figure 2b), when only soybean oil was used, 8% of lipase was required to reach 96.5% yield; although, the minimum amount of lipase required to reach 96.5% increased as the content of waste cooking oil in the mixture increased. When only waste cooking oil was used, around 14% in mass of lipase were added to reach the minimum yield established for it to be considered biodiesel. When the reaction time was reduced to 4 h (Figure 2c), a larger amount of biocatalyst was required. However, when 15% of Novozyme 435 was added to the reaction medium, yields higher than 96.5% were reached, regardless of the type of mixture of oils used.

After this analysis, we can conclude that, regardless of the reaction time, when high amounts of biocatalysts are added to the reaction medium, the type of the oil mixture does not interfere in the methyl ester yield. Considering the experiment done in 4 h of reaction, time that is more reasonable for it to be used in industrial applications, it was possible to reach the minimum yield established by ANP using a mixture containing 50% of waste cooking oil and 14% of Novozyme 435. For the same reaction time and oil mixture, it was also possible to reach a yield of 98.5% using 20% of lipase. In contrast, when pure waste cooking oil was used in a reaction of 4 h, at the most interesting conditions for use in industrial applications, the minimum amount of lipase to reach a yield of 96.5% was 15%. For systems with more than 15% of lipase added to the medium, each additional 2% of lipase added leads to an increase of 2% in the yield. Thus, it was possible to reach a yield of 99.2% using 20% of Novozyme 435 and waste cooking oil.

The study showed that it is possible to achieve high conversions at low reaction times and using different soybean and waste cooking oil mixtures. For this, a greater amount of lipase would be required. However, it is important to highlight that lipases can be reused in batch processes and, therefore, the study of its stability in the reaction medium is of great importance.

### 3.2 Enzyme reutilization

A study of the stability of the Novozyme 435 was conducted with the same conditions used for the experiment number 14 (12.5% of lipase, 50% of WCO in the mixture, and 10 h of reaction), which is the treatment that provided the greatest yield. The lipase was used repeatedly during 5 cycles.

After being reused for 5 cycles, the lipases maintained 93.9% of their initial activity. There was a reduction in the methyl ester yield from 97.2% to 91.3% after 5 cycles, as can be seen in Figure 3. From the results, one can conclude that Novozyme
435 can be reused repeatedly in this reaction medium without causing a significant activity reduction. This result corroborates others reported in the literature (Hernández-Martín & Otero, 2008; Zheng et al., 2009; Gharat & Rathod, 2013), which confirm Novozym 435 as a promising biocatalyst for industrial applications, since it has a great operation stability.

### 3.3 Determination of kinematic parameters

The kinetic was studied based on an experiment done at a constant agitation speed of 400 rpm, molar ratio of 3:1, and 12.5% of lipase at 45 °C. Although the kinetic models had been used for other systems without the presence of enzymes, the results indicated that they could represent the transesterification process well, being simpler and producing a rapid estimation. Moreover, less experimental variables are necessary, minimizing experimental costs.

#### 3.3.1 Power-law model

After plotting the experimental data, we fitted it into the power law model, and Eq. 14 was obtained. The graph of experimental data and predicted yield by model is presented in Figure 4. The kinetic parameters obtained were 2.1045 mol$^3$ L$^3$ h$^{-1}$ for the direct overall reaction, and 0.0002 mol$^3$ L$^3$ h$^{-1}$ for the reverse overall reaction. The parameters show that the direct reaction has a higher influence on biodiesel yield.

$$ r = 2.1045 \times [TG] \times [ROH]^3 \times 0.0002 \times [BD]^3 \times [GL] \quad (14) $$

The coefficient of determination ($R^2$) for this model was about 0.997, which indicated a good correlation to experimental data.

#### 3.3.2 Eley-Rideal mechanism

Eq. 15 represents the model fitted to experimental data, and the graph is illustrated in Fig. 5.

$$ r_s = \frac{1.6040 \times C_{TG} \times C_{ROH}^3 \times 0.0008 \times C_{GL} \times C_{FAME}^3}{(1 + 0.1505 \times C_{TG} + \frac{1}{7.5975} \times C_{GL})} \quad (15) $$

The determination coefficient ($R^2$) obtained from this model is 0.998. The kinetic parameters estimated are 1.6040 mol$^3$ L$^3$ h$^{-1}$ for the direct superficial reaction, 0.008 mol$^3$ L$^3$ h$^{-1}$ for the reverse superficial reaction, 0.1505 mol$^3$ L$^3$ h$^{-1}$ for the adsorption of triglycerides, and 7.5975 for the desorption of glycerol. As observed for the Power Law model, the direct reaction presented a greater significance in the final yield than the inverse reaction. In regards to adsorption and desorption reactions, one can observe that the desorption...
The two models could represent well the experimental yields as a function of time since they had high determination coefficients. More than the determination coefficients, the methods were also compared based on squared errors summation (Table 6). Therefore, one can conclude that the best model to describe this enzymatic transesterification is the Eley-Rideal. Al-Sakkari et al. (2017) and Chantrasa et al. (2011) also compared the power law model with the Eley-Rideal mechanism, observing that the Eley-Rideal mechanism provided the better description of the biodiesel production.

4. CONCLUSIONS

In the present study, we identified the technical viability of using Novozyme 435 as catalyst due to the high yields obtained. The highest yield reached 98.04% using an oil mixture containing 50% of WCO, 12.5% of Novozym 435, and under 10 h reaction. The study indicates that lipase dosage is the most significant variable affecting the yield. The increase in lipase amount improves the methyl ester yield. The commercial lipase studied revealed a great operational stability after 5 cycles of repeated usage, and the model of Eley-Rideal was suitable to represent the biodiesel production.

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5. REFERENCES


