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Cleaner Production of Isopropyl Laurate using Lipase Catalyzed Esterification: Optimization by Response Surface Methodology

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Abstract

This work aims to produce a common emollient ester isopropyl laurate (IPL) in a solvent-free system. Isopropyl alcohol (IPA) and lauric acid (LA) were esterified in a closed batch reactor, employing immobilized *Candida antarctica* lipase as a biocatalyst. Response surface methodology was based on a five-levels, three variables composite design was used to optimize the reaction conditions. The interactive effect of three different parameters on isopropyl laurate (IPL) synthesis was studied. The following conditions' ranges were considered: molecular sieves of 1%–10% (w/w), IPA-to-LA molar ratios of 3:1–15:1, and enzyme load of 1%–4% (w/w). As a result of the optimization study, the optimum conditions were 4% w/w of Novozym 435, 15:1 IPA: LA molar ratio, and 10% w/w molecular sieves at 150 RPM, 60°C and for 2.5 h. The RSM study showed that the maximum predicted and experimental conversion values were 90.75 and 91%, respectively. It is worth mentioning that Novozyme 435 demonstrated superior operational stability, where it was used for 15 cycles without significant denaturation. The clean nature of the proposed method and the proven operational stability of Novozym 435 reveal this approach's technical and economic feasibility.

Keywords: Sustainable development goals, Isopropyl Laurate, Environment, Cleaner production, Esterification

1. Introduction

Currently, research focuses on using green specialty esters as binders, perfumes, antistatic agents, lubricants and emollients (1). These specialty esters are expensive but also have large profit margins (2). Isopropyl laurate (IPL) is a common specialty ester that possesses many applications. Furthermore, because it's non-greasy, IPL has several uses in cosmetics. In addition, it can be used to manufacture various end products, including deodorants, pressed powders, creams, lotions, and bath oils. Finally, it has numerous uses, including as a co-solvent and solvent in the paint and ink industries (3).

Enzymatic and chemically catalyzed processes are used to esterify lauric acid (LA) and isopropyl alcohol (IPA) to create IPL (4). Industrially, IPL is manufactured using a well-established chemical process; this method is well recognized and has received widespread industrialization (1). This method relies on fatty acids being esterified using a typical chemical catalyst such as mineral acids, tin salts, organo-titanates, silica gel, or cation exchange resins at elevated temperatures between 180°C and 220°C (5). To enhance the chemical process further, reactive distillation has recently been used (6). Such systems combine the functions of chemical reaction and distillation into one piece of machinery (7). This method does away with the product's further purification and processing (8). Although this approach offers many benefits, its implementation is constrained by its high initial cost and many environmental issues (9). Additionally, the use of high reaction temperatures in the chemical process results in the production of materials that are discolored (10, 11).

Numerous authors have also investigated the synthesis of esters using active heterogeneous catalysts. For example, Amberlyst 15 was used as a heterogeneous catalyst to catalyze the production of methyl palmitate at 70°C in a semi-batch reactive distillation column. Aqar et al. (12) observed a high conversion of 99% in this context. Furthermore, Mutlu and Yilmaz (13) discovered that the SBA-15 catalyst contributed to converting 63% of palmitic acid to cetyl palmitate. Previous research suggested that palmitate and laurate esters might be produced chemically at low temperatures. However, heterogeneous catalysts are expensive, which may limit their use on a broad scale (14). Utilizing lipase-catalyzed approach offers many advantages over the conventional chemical process, such as it consume less energy (15) and produces improved product quality (16).

According to the literature, considerable esterification reactions have been studied utilizing various solvents, including tert-butanol, heptane, and hexane, to enhance reaction medium homogeneity. However, these solvents make downstream processing more difficult due to several purification stages. Therefore, solvents are not preferred because the targeted product of IPL, in this case, has a principal application in the cosmetics industry (17).

Additionally, a reaction driving force must be present for an enzymatic reaction with high conversion to proceed in a forward direction in a single-phase medium (18). Based on that, one of the key factors influencing enzyme activity is the water concentration. Therefore, shifting the reaction equilibrium into IPL synthesis is more advantageous by reducing the water content produced during the reaction course. It should be noted that

lipase activity declines below a particular water concentration due to enzyme dehydration (19). As a result, maintaining high reaction conversion requires an ideal water content. In general, it is important to separate the water produced during the reaction; typically, it can be done by vacuum distillation (20) or utilizing molecular sieves (21). Molecular sieves can reduce water byproducts without a solvent (22). However, too many molecular sieves might inhibit the interactions between the enzyme and substrate, slowing down the reaction rate by adsorbing the necessary water.

Typical optimization entails keeping other fixed conditions constant while changing one condition simultaneously. However, this strategy requires multiple runs (23). Additionally, because it is one-dimensional, this approach frequently does not ensure the identification of ideal conditions (24). Here, the reaction conditions were optimized using the response surface methodology (RSM). RSM is useful for analyzing complicated processes by running a few chosen tests (25). Furthermore, such a design offers minute particulars and a thorough justification of the reaction (26).

The present work intends to study the enzymatic production of isopropyl laurate in a solvent-free system using a clean approach. *Candida antarctica* lipase would replace the chemical catalyst's need, resulting in reduced significant energy. The present work supports SGD No. 12, highlighting responsible production and consumption.

2. Materials and Methods

A stoppered batch reactor carried out the esterification reaction between lauric acid and isopropyl alcohol. For each run, a reaction mixture total mass of 10 g was fed to the rector. All experiments in this study were performed in triplicate.

2.1. Materials

Novozymes provided the immobilized Candida antarctica lipase (Novozym 435). (Denmark). A CALB lipase immobilised on a hydrophobic carrier is Novozym® 435. *Candida antarctica* lipase B is a non-specific lipase called CALB. Other chemicals such as molecular sieves, 8–12 mesh of beads, acetone, isopropyl alcohol, and lauric acid > 99%, were purchased from Sigma Aldrich (St. Louis, MO, USA). All other reagents were all of the analytical variety.

2.2. Time course of IPL production

The esterification reaction time between IPA and LA was examined. The enzymatic esterification reaction was performed using the following conditions: 15:1 IPA/LA molar ratio, 150 rpm agitation rate and 60 °C temperature. The esterification batch reactor was shaken and heated by a water bath shaker. Two separate reactions were carried out: (1) by adding 10% w/w of molecular sieves to the reaction, and (2) no molecular sieves were introduced. Aliquots were periodically taken out every 30 minutes for up to 3 hours. By

titrating against 0.1 M of NaOH, the residual fatty acids in the reaction mixture were calculated.

2.3. IPL production

LA and IPA were esterified using a 50 mL batch reactor (a closed stoppered reactor) in the presence of a lipase. Novozyme 435 ranged from 1% to 4% w/w of the total mass of reactants. A range from 3:1 to a 15:1 alcohol to lauric acid molar ratio was examined. In addition, a range of 1%–10% w/w of molecular sieves was investigated. Every 30 minutes, 200 microliters of aliquots were taken out. Further, 10 mL of acetone/ethanol mixture (50:50, v/v) was added to stop the reaction from progressing. Control experiments without the use of immobilized enzymes were carried out. Calculating the amount of free LA in the reaction mixture allowed us to determine the conversion percentage. Phenolphthalein was used as an indicator, and 0.1 M of NaOH solution as a titrant. Free fatty acids (FFA) were neutralized to the endpoint and calculated as per Eq. 1:

$$\text{Conversion (\%)} = \frac{\text{N} - N^{o}}{N} \text{ x } 100, \quad (1)$$

where N° represents the amount of NaOH consumed after adding Novozym 435, and N represents the amount of NaOH consumed without adding Novozym 435. A maximum experimental error of 2.5% was calculated, while a minimum error of only 0.5% was found.

2.4. Experimental design

In this work, IPL synthesis was studied by studying the interactive effects of three parameters. A complete three-factor design, fractional five-level, and twenty runs in total were suggested. To evaluate the yield of IPL synthesis, three factors were chosen: the amount of molecular sieve (1%-10%), IPA/LA molar ratio (3:1-15:1), and the quantity of lipase (1%-4%, w/w). Notably, this reaction was carried out without the use of any solvent, and the greatest molar ratio of IPA to LA was chosen to be 15:1. The minimal molar ratio for IPA/LA to keep the reaction mixture's viscosity at a tolerable level was determined to be a 3:1 ratio. To maintain a high conversion, a minimum percent 1% (w/w) of Novozym 435 was chosen, it was seen that further lower enzyme load percent results in significantly lower conversion. In contrast, 4% (w/w) of enzyme amount was chosen as the highest amount of Novozym 435 that could retain the suggested process's economic viability. The collected data were modeled using a quadratic equation (Eq. 2).

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{\substack{i=1 \\ i \le j \le 3}}^{3} b_i b_j x_i x_j, \quad (2)$$

where the letter Y indicates the response, xi is the independent variable, and bi, bj, and bo, are the constant coefficients. Utilizing Design Expert Software, response surfaces, analysis

of variance (ANOVA), and regression studies were conducted (Version 13.0.3). The ideal reaction parameters were generated using equations 2 and 3.

2.5. Operational stability of Novozym 435

Investigations were made into Novozym 435's operational stability using the suggested method. With 10 g of reactants, the reaction was carried out in a closed batch reactor. The following process parameters were chosen: 60 °C, 150 rpm, 4% weight-weight Novozym 435, 10% w/w molecular sieve quantity and 15:1 IPA/LA molar ratio. Filtration was used to remove the molecular sieves and enzyme after the reaction was finished, IPA was then used to wash the enzymes before utilizing them for the subsequent batch.

3. Results and Discussion

3.1.Time course of the enzymatic production of isopropyl laurate

The most appropriate reaction time for IPL production using Novozym 435 was studied in two cases (with and without molecular sieves). Fig. 1 shows that in the absence of molecular sieves case, after passing only 30 min, the conversion steadily increased to 65%. Further increase in the conversion was observed where 74% of lauric acid was converted to IPL after 2.5 h. However, in the case of adding molecular sieves, the conversion obtained after passing the first 30 min of the reaction time was 72%. The conversion value then gradually increased over the course of the next 2.5 hours, peaking at 89.5%. It is worth mentioning that after 2.5 h, no more increase in the conversion was observed in both cases. Based on the previous, the reaction time of 2.5 h in the presence of molecular sieves was selected for all experiments.

Numerous articles have examined the impact of including molecular sieves in the esterification reaction. By preventing the buildup of water throughout the reaction, these publications consistently demonstrate the beneficial effects of moving the equilibrium of the reaction in the path of esterification instead of hydrolysis (27, 28).

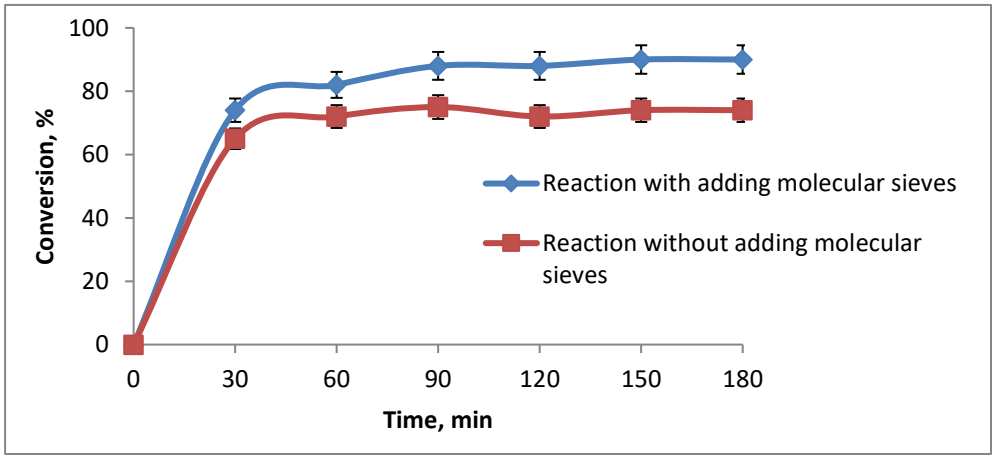


Figure 1. Effect of molecular sieves addition on the enzymatic conversion of lauric acid and isopropyl alcohol to isopropyl laurate. Reaction conditions: (molar ratio of substrate (palmitic acid: isopropyl alcohol 1:15, amount of enzyme: 4% (w/w, agitation speed: 150 rpm, and Reaction temperature: 60 ℃). Arrow bar indicates standard deviation of triplicate.

3.2. ANOVA

Design Expert Software (Version 13.0.3) was employed to optimize the esterification reaction conditions. The results of ANOVA showed that the proposed model was determined to be significant based on its low p-value (0.05) and high F-value. Furthermore, the associated model variable with a low p-value (0.05) was considered to have the most significant impact model equation (24, 25). The experimental and predicted outcomes of IPL generation with Novozym 435 are displayed in Table 1. The table demonstrates that the residual values are quite small. The normal distribution of error is indicated by this finding, which also points to an agreement between the experimental and predicted values. In the present study, an F-value of 666.01 represents that the proposed model implies significance, as shown in Table 2. Furthermore, the suggested model has a high coefficient of determination of R2 = 0.9983, as indicated by its high confidence level of 99.9% and its total variation of 0.1%. Therefore, other minor factors may affect the conversion value of the esterification reactor.

Table 1. Experimental versus predicted results of lipase catalyzed production of isopropyl laurate

Order	Novozym 435 %, (w/w)	Molar Ratio	Molecular Sieves %, (w/w)	Experimental Value	Predicted Value
1	4	3	1	80	79.6
2	2.5	9	0	83	83.87
3	2.5	9	5.5	86	85.37
4	1	3	1	55	54.26
5	5	9	5.5	87	87.2
6	4	15	1	90	89.77

7	2.5	9	5.5	85	85.61
8	2.5	-1	5.5	60	60.21
9	1	3	10	59	58.59
10	2.5	9	13	88	88.03
11	1	15	1	70	69.04
12	2.5	9	5.5	84.80	85.37
13	0	9	5.5	50	50.88
14	1	15	10	73.00	73.76
15	2.5	9	5.5	85.90	85.37
16	4	15	10	91.00	90.75
17	2.5	9	5.5	85.20	85.37
18	2.5	9	5.5	85.50	85.37
19	4	3	10	80.5	80.83
20	2.5	19	5.5	80	80.69

Table 2: Analysis of variance of isopropyl Laurate production

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	*p*-Value Prob > F
Model	2945.02	9	327.22	666.01	<0.0001
A-Molar ratio	506.09	1	506.09	1030.05	<0.0001
B-Enzyme amount	1576.40	1	1576.40	3208.47	<0.0001
C-Molecular sieve	20.94	1	20.94	42.61	<0.0001
AB	9.03	1	9.03	18.38	0.0016

AC	0.0313	1	0.0313	0.0636	0.806
BC	3.78	1	3.78	7.7	0.019
A^2	401.24	1	401.24	816.65	<0.0001
B^2	485.89	1	485.89	989	<0.0001
C^2	0.590	1	0.590	1.21	0.296
Residual	4.91	10	0.4913		
Corrected total	2949.93	19			

3.3. Regression analysis

The data sets were then subjected to regression analysis in order to get relevant formulated equations and coefficients that can represent the model properly. In addition, the range of the expected values at the average prediction error and the design points were compared to determine adequate precision. The current model's rating of 80.441 indicates that it can be utilized to explore the design space. Desirable models are suited for a value greater than 4. Finally, in order to examine the accuracy and dependability of the carried-out trials, the variation coefficient is calculated. In this work, a variation coefficient of only (6.89%) was obtained, highlighting the presented results' accuracy.

It should be highlighted that the high F-value represents the significance of the model terms. For example, table 2 shows that an F-value of 1030 was obtained for the IPA/LA molar ratios. That value was found to be lower than that of the Novozym 435 quantity variable's F-value of 3208. This finding suggests that the quantity of Novozym 435 may be a key factor influencing the conversion value. Based on the aforementioned values, an empirical equation was created to estimate the response value and uncoded screen variables at any time.

$$Y = +85.37 + 6.09A + 10.74B + 1.24C - 1.06AB - 0.0625AC -$$

$$0.6875BC - 5.28A^2 - 5.81B^2 + 0.2035\,C^2 \quad (3)$$

where Y stands for conversion, while A represents the amounts of Novozym 435, B is IPA/LA molar ratio, and C is molecular sieve.

3.4. Response Surface Plots

3.4.1. The effect of Novozym 435 load and molecular sieves amount on conversion

Figure 2a shows the impact of the amounts of Novozym 435 and molecular sieves on the conversion of IPL at 2.5 h (optimum reaction time). The response surface plot's interaction generated the best outcomes. According to the graph, high molecular sieve

percentages, high Novozym 435 amounts, low molecular sieve percentages and high enzyme concentrations could produce a high response. Such a finding highlights that Novozym 435 amount considerably affects the conversion value. According to Fig. 2a, the conversion achieved at 1% (w/w) of molecular sieves and 4% (w/w) of Candida antarctica lipase is comparable to that achieved at 10% (w/w) of molecular sieves and 4% (w/w) of *Candida antarctica* lipase at a molar ratio of 9:1 isopropanol-to-LA. Lin (2003) (29) has reported similar findings on the correlation between the quantity of immobilized enzyme and the molecular sieve percentage.

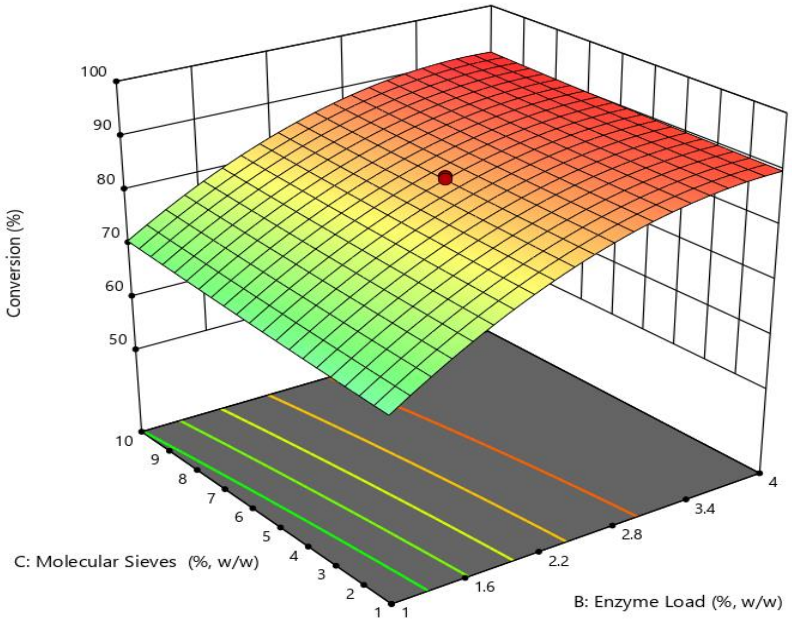
3.4.2. Interaction between molar ratio and molecular sieves

Figure 2b shows the response surface plot on the IPL synthesis utilizing Novozym 435 at 2.5 hours as a function of the molar ratio and molecular sieve. The interaction response surface plot was created at a fixed Novozym 435 quantity of 2.5% (w/w). According to the data depicted in the figure, conversion rises with an increase in the IPA/LA molar ratio at either a high or low molecular sieve percentage. High conversion of the process was encouraged by molecular sieves of 10% w/w and a 4:1 molar ratio of isopropanol to LA. The esterification process equilibrium is shifted toward completion at high molar ratios of alcohols to fatty acids (30) (31).

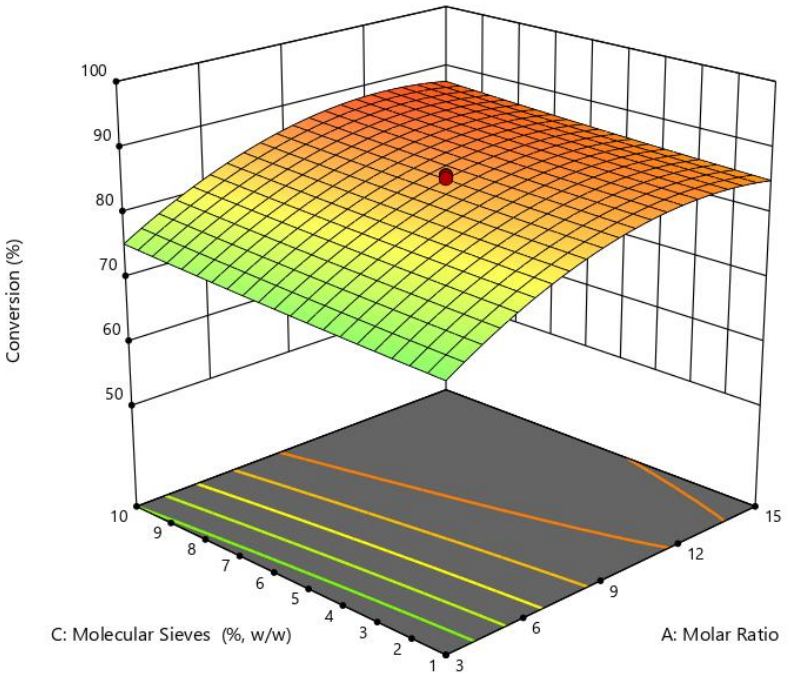
3.4.3. The interaction between the amount of Novozym 435 and the molar ratio

The effect of the Isopropanol/LA molar ratio and Novozym 435 load on the generation of IPL is represented as per Fig. 2c. The surface plot of the reaction was produced for the ideal values. The graph demonstrates that conversion rises as the quantity of Novozym 435 and molar ratio rise. For example, the amount of Novozym 435 (4% w/w) and the molar ratio of 15:1 for isopropanol to LA yield a conversion of 91%, which reveal that the latter conditions are optimal for the catalytic synthesis of IPL. On the other hand, a sharp decline in the conversion was approached at 1% (w/w) of Novozym 435 and a 1:1 molar ratio. The low conversion in this study, at about 60%, was the lowest value ever observed. This result showed that the conditions of the quantity of Novozym 435 and isopropanol/LA molar ratio were relevant, although the proportion of molecular sieves was less so.

According to the current research and RSM, the ideal conditions are as follows: Novozym 435 (4% weight-to-weight, 0.4 g), 60°C, 10% weight-to-weight molecular sieve (1 g), and a 15:1 isopropanol/PA molar ratio (7.78 g IPA + 2.22 LA). A maximum conversion of 91% was achieved.


Conversion (%)
100
90
80
70
60
50
C: Molecular Sieves (%, w/w)
10
9
8
7
6
5
4
3
2
1
B: Enzyme Load (%, w/w)
1
1.6
2.2
2.8
3.4
4

(a)


Conversion (%)
100
90
80
70
60
50
C: Molecular Sieves (%, w/w)
10
9
8
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3
2
1
A: Molar Ratio
3
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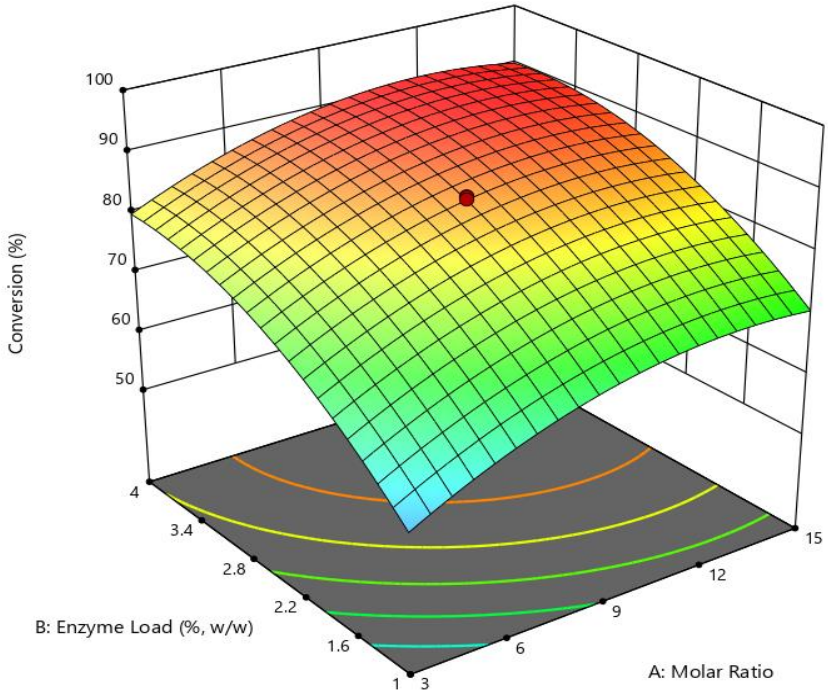
9
12
15

(b)



(c)

Figure 2. Response surface figures. (a) Novozym 435 Amount vs load of molecular sieve. (b) Molar ratio vs the number of molecular sieves. (c) Molar ratio compared to Novozym 435 dosage.

3.5. Operational stability of Novozym 435

The high cost of commercial enzymes represents the biggest obstacle to the commercialization of the enzymatic process. Although this technique has demonstrated its viability in a few applications, including margarine and biodiesel, such green technology has still not yet been extensively applied by the oleo chemical industry. As a result, expensive enzymes must be used as effectively as possible for a process to be economically viable.

One strategy to support the process economy is the reuse of lipase. Several cycles of lipase reuse have been attempted in numerous earlier articles [35-39]. In this study, 15 cycles were used to assess Novozym 435's operational stability (Fig. 3). After every cycle, filtration was used to separate Novozym 435, and the filtered IPL was washed by IPA. After washing the immobilized lipases, they were dried and introduced again to catalyze other esterification reactions.

Here, the operational stability was calculated by dividing cycle n's conversion by cycle 1's. According to this study, cycle one's operational stability equals 100%. At 60°C, the operational stability investigation was carried out while retaining the other ideal conditions. This study demonstrated that after being used for 15 cycles, Novozym 435 still exhibited more than 95% of its initial activity. Similar outcomes were similarly reported by Lin et al. (29) or the lipozyme RM IM-catalyzed synthesis of palm esters in a stirred tank reactor. Also, Zhong et al. reported similar results for the 1,3-diacylglycerol enzymatic production in a solvent-free environment utilizing lipozyme RM IM (32).

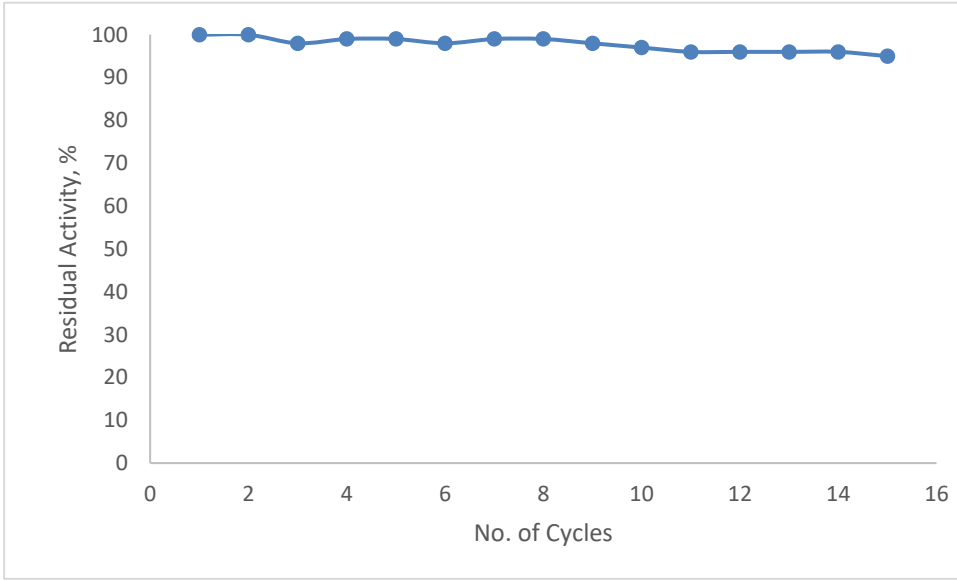


Figure 3. Operational stability of Novozym 435. Conditions (15:1 isopropyl alcohol-to-palmitic acid molar ratio, 60 °C, 150 rpm, 4% w/w enzyme, and 10% w/w molecular sieve quantity).

Process economy and the number of enzyme reusability cycles are inversely correlated when the enzymatic approach is used for esters production. The process is considered economically viable when a product's market price exceeds its overall production cost. Mustafa et al. (15) had already looked into the commercial potential of glyceryl monolaurate manufacturing. According to the authors, the immobilized lipase can

be reused up to 50 times before the economic viability is no longer viable. Additionally, they demonstrated how the same outcome could be achieved by reducing the enzyme dose from 2.5 kg/t to only 0.5 kg/t while preserving ten cycles of reusability. 2-ethyl hexyl oleate was produced using Novozym 435 by Hosney and Mustafa, (8), the authors have developed a thorough economic analysis in a fixed bed reactor. According to the authors, Novozym 435 offered a 2 t/kg productivity. They concluded that their enzymatic method was 30% profitable concerning the product market price. In the current work, to examine the economic feasibility, the author is carrying out the proposed reaction in a fixed bed reactor. Future works by the author will include this information.

4. Conclusion

In the current study, Novozym 435 served as a biocatalyst in a solvent-free system for the esterification of IPA and LA. The optimum conditions were as follows: 60°C temperature; 4% (w/w) Novozym 435 amount, 15:1 IPA-to-PA molar ratio; 10% (w/w) molecular sieve percentage, and 2.5 hours reaction duration. The maximum conversion achieved was as high as 91%. In addition, Novozym 435 kept more than 94% of its initial activity even after 15 batches of use. Lipases appeal more to consumers than those produced chemically because of the environmental advantages of the enzymatic approach (particularly in food and cosmetic applications).

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