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Green synthesis of isopropyl palmitate using immobilized *Candida antarctica* lipase: Process optimization using response surface methodology

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ABSTRACT

This work aims to produce isopropyl palmitate (IPP), a common emollient ester in a solvent-free system. An esterification reaction between isopropyl alcohol (IPA) and palmitic acid (PA) was performed in a closed batch reactor using immobilized *Candida antarctica* lipase as a biocatalyst. Reaction conditions were optimized using response surface methodology based on a five-level, three-variable composite design. The interactive effects of conditions on the IPP yield were investigated in the following ranges: IPA-to-PA molar ratio of 3:1–15:1, 1%–4% (w/w) Novozym 435, and 1%–10% (w/w) molecular sieves. The optimum conditions were IPA-to-PA molar ratio of 15:1, 4% w/w of Novozym 435, and 10% w/w of molecular sieves at 60°C and 150 RPM for 2.5 h. The maximum experimental and predicted conversion values were 90.00% and 90.92%, respectively. Moreover, Novozym 435 exhibited remarkable operational stability because it was used for 15 cycles without considerably losing its original activity. In studying the feasibility of the proposed method, a process flow diagram was suggested to perform the semicontinuous production of IPP in a solvent-free medium.

1. Introduction

At present, using green specialty esters as antistatic agents, binders, emollients, fragrances, and lubricants has been a research of interest; (Mustafa et al., 2016a). Such fine esters have high prices but also high profit margins (Hoseny and Mustafa, 2020). Isopropyl palmitate (IPP) has numerous applications in cosmetics industry because of its nongreasy nature. Further, it has various applications in producing bath oils, creams, lotions, make-up and hair-care products, deodorants, and pressed powders. Finally, it has versatile applications in areas such as a solvent or cosolvent in ink and paint industries (Abdelmoez and Mustafa, 2014).

IPP is produced by esterifying palmitic acid (PA) with isopropyl alcohol (IPA) using enzymatic and chemically catalyzed routes (Rajendran et al., 2009). Commercially, IPP is synthesized using a well-established chemical method; such approach is well known, and it has been industrialized globally (Hoseny and Mustafa, 2020). This approach depends on fatty acids esterification at elevated temperatures in the range of 180°C–220°C using conventional catalyst such as tin salts, mineral acids, organo-titanates, cation exchange resins, or silica gel (D'Ambrosio et al., 2021). Recently, reactive distillation has been applied to improve the chemical process (Ibrahim and Mustafa, 2022). In such systems, distillation and chemical reaction are performed in a single equipment (Mustafa et al., 2022). This recent technique eliminates the product downstream purification/processing (de Jong, 2010). Despite considerable advantages offered by this method, its application is hindered owing to its high investment cost and many environmental issues (Tsouko et al., 2021). Moreover, a chemical method obtains discolored materials (Mustafa et al., 2016b) because of high reaction temperatures used (Abdelmoez et al., 2013).

Using active heterogeneous catalysts to synthesize palmitate esters has also been studied by many authors. Aqar et al. (2021) reported a high conversion of 99% for methyl palmitate production in a semibatch reactive distillation column using Amberlyst 15 as a heterogeneous catalyst at a low temperature of 70°C. Furthermore, Mutlu and Yilmaz (2016) reported that cetyl palmitate with a conversion of 63% could be produced using W-loaded and Zr-incorporated SBA-15 catalysts. Previous studies reported the possibility of producing palmitate esters using a chemical technology at low temperatures. However, the high cost of heterogeneous catalysts (Inayat et al., 2021) may restrict their large

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scale applications.

Using enzymes for catalyzing an esterification reaction may provide many advantages over the chemical method (Hoseny et al., 2020a). These advantages include improved product quality, low energy consumption, and clean and environmentally friendly production route (Hoseny et al., 2020b). Novozym 435 is an industrially immobilized lipase manufactured by Novozymes. The immobilization system depends on the interfacial activation of lipase B from *Candida antarctica* on a resin, such as Lewatit VP OC 1600. This resin is a macroporous support formed by poly(methyl methacrylate) crosslinked with divinylbenzene. Novozym 435 is a widely used commercial biocatalyst in academy and industry (Zhong et al., 2013).

Based on literature, many esterification reactions have been investigated using different solvents, such as heptane, hexane, and *tert*-butanol, to improve reaction media homogenization. However, these solvents complicate the downstream processing because of long purification steps (Petersson et al., 2005). Herein, the targeted product of IPP has a primary application in the cosmetic sector; thus, using solvents is not preferable (Freitas et al., 2007)

Moreover, for a high conversion enzymatic reaction occurring in a single-phase medium, a reaction driving force should exist to propel the targeted reaction in the forward direction (Won and Lee, 2001). Therefore, water content is a main parameter affecting the enzyme activity. Decreasing the water content generated during the reaction favors shifting the reaction equilibrium into IPP synthesis. Notably, below a specific water content, the lipase activity decreases due to enzyme dehydration (Mustafa, 2021). Consequently, an optimum water content plays a crucial role in maintaining high reaction rates. In general, separating water generated during the reaction is necessary; commonly, it can be removed using vacuum distillation (Aguieiras et al., 2011) or molecular sieves (Li et al., 2011). In a solvent-free system, molecular sieves can eliminate water byproduct (Silva et al., 2015). However, excess molecular sieves can negatively affect the reaction rate by adsorbing the essential water, thereby restraining the interactions between the substrate and enzyme (D'Ambrosio et al., 2021).

Conventional optimization involves differing one condition at a time and maintaining other fixed conditions (Mostafa et al., 2013). However, this approach needs several runs (Salleh et al., 2016). Further, this approach often does not guarantee the determination of optimal conditions because it is single dimensional (Abdelmoez et al., 2016). Here, the response surface methodology (RSM) was used to optimize the conditions of the reaction. RSM is an effective approach to study complex processes by performing a few selected runs (Gunawan et al., 2005). Such a design provides fine details and the complete explanation of the reaction (Haider Ali et al., 2015).

This work aims to study the technical feasibility of producing IPP based on the principles of green chemistry through the direct enzymatic esterification of PA and IPA using the immobilized *Candida antarctica* lipase (Novozym 435) in a single-phase system. Moreover, a plant design based on the semicontinuous production of IPP in the form of a process diagram was suggested to address the reliability of this method on a commercial scale.

2. Materials and methods

In the current work, a closed batch reactor was used in IPP esterification. The same total mass of reactants (PA plus IPA) of 10 g was considered for all runs. All experiments were tested in triplicate, and the mean values represented the values of conversion.

2.1. Materials

Immobilized *Candida antarctica* lipase (Novozym 435) was donated by Novozymes (Denmark). Novozym® 435 is a CALB lipase immobilized on a hydrophobic carrier (acrylic resin). CALB is a nonspecific lipase originating from *Candida antarctica* B. Isopropyl alcohol, PA > 99%, acetone, 4 Å of ethanol and molecular sieves, and 8–12 mesh of beads were purchased from Sigma Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Time course of IPP production

Two-time progress curves were developed to investigate the optimum reaction time of the proposed enzymatic reaction between IPA and PA and to synthesize IPP. In both cases, the reaction conditions were as follows: IPA/PA molar ratio of 15:1, temperature of 60°C, and agitation speed of 150 rpm. The reaction was performed in a batch reactor heated and agitated using a water bath shaker. In the first case, 10% w/w of the total mass of the reactants of molecular sieves was added, whereas in the second case, the reaction was conducted without adding molecular sieves. Periodically, aliquots were withdrawn every half an hour for up to 3 h. The remaining fatty acids in the mixture of the reaction were estimated via titration against 0.1 M of NaOH.

2.3. IPP production

The esterification reactions between PA and IPA were conducted in a closed (with a stopper) conical flask with 50 mL of Novozym 435 containing 1%–4% w/w of the total mass of reactants (this amount corresponds to the amount of enzyme plus support). The IPA and PA molar ratios ranged from 3:1 to 15:1. Molecular sieves were added in the range of 1%–10% w/w. The esterification reaction was performed in a shaking water bath at 60°C at 150 rpm. Two hundred microliters of aliquots was withdrawn every 30 min. Further, 10 mL of (50:50, v/v) acetone:ethanol blend was added to terminate the progress of the reaction. Control runs were performed without using immobilized enzymes. The conversion percentage was estimated by calculating the remaining free PA in the mixture of the reaction. 0.1 M of NaOH solution was used as titrant to neutralize free fatty acids (FFA) to the end point using phenolphthalein as an indicator. The conversion values were estimated using Eq. (1).

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

where N denotes the volume of NaOH consumed without Novozym 435 addition, and N° denotes the volume of NaOH consumed with Novozym 435 addition.

All runs were tested in triplicate via titration, and the values of conversion were represented with regard to mean calculation. The minimum experimental error was in the range of the mean value of ±0.5%, whereas the maximum mean value of the experimental error was in the range of ±2.5%.

2.4. Experimental design

In this study, RSM comprising a full fractional five-level, three-factor design was used to investigate the interactive effects of three parameters on IPP synthesis. A total of 20 runs were proposed. Three variables were selected to study the yield of IPP synthesis, i.e., IPA/PA molar ratio (3:1–15:1), lipase amount (1%–4%, w/w), and molecular sieve amount (1%–10%). Notably, this reaction was performed without solvent addition, and a 15:1 IPA/PA molar ratio was selected as the highest molar ratio. An IPA/PA molar ratio of 3:1 was selected to be the minimum molar ratio to maintain the reaction mixture at a moderate viscosity. 1% (w/w) of Novozym 435 was selected as a low-moderate amount; a low percentage value could remarkably reduce the conversion. Conversely, 4% (w/w) of Novozym 435 was selected as the maximum possible percentage to maintain the economic feasibility of the suggested process. The data obtained were fitted into 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 Y denotes the response; x_i denotes the independent variable; and b_o, b_i, b_{ii}, and b_{ij} denote the fixed coefficients. Consequent analysis of regression, response surfaces, and analysis of variance (ANOVA) were performed using Design Expert Software (Version 9.0.3). Equations (2) and (3) were used to generate the optimal reaction parameters.

2.5. Operational stability of Novozym 435

The operational stability of Novozym 435 under the proposed method was investigated. The reaction was conducted in a closed batch reactor with 10 g of reactants. The following process conditions were selected: 4% w/w Novozym 435, 15:1 IPA/PA molar ratio, and 10% w/w molecular sieve amount at 60°C and 150 rpm. After the reaction completed, enzyme and molecular sieves were removed using filtration and then they were washed with IPA for the next cycle.

3. Results and discussion

3.1. Effect of reaction time

The enzymatic synthesis time course of IPP using Novozym 435 has been investigated with and without using molecular sieves. Fig. 1 shows that the percentage of conversion increased steadily to approximately 66% in a short reaction time of 30 min in the absence of molecular sieves. In this case, the maximum conversion of 74% was achieved at a reaction time of 2.5 h. Moreover, using molecular sieves, more than 70% of the PAs were esterified in the first 30 min. Afterward, the conversion value progressed slowly with time until its maximum value of 90% was achieved at the same time of 2.5 h. After the reaction time of 2.5 h, no significant increase in the percentage of conversion was observed. Based on this result, molecular sieves were used in all runs to study the optimization and operational stability under varying conditions. Furthermore, the reaction time of 2.5 h was selected for all runs.

The effect of adding molecular sieves to the esterification reaction has been discussed in many papers. These papers show consistent results on the positive effect of shifting the reaction equilibrium toward esterification rather than hydrolysis by avoiding the accumulation of water during the reaction (Kavadia et al., 2017; Vadgama et al., 2015).

3.2. ANOVA

Design Expert Software (Version 9.0.3) was used to determine the predicted values of conversion. The results of ANOVA suggested that based on the proposed model high F-value and low p-value (<0.05), it was approved to be significant. Moreover, a model variable having low p-value (<0.05) was shown as the term having the highest effect on the related model equation (Kutlu and Kocar, 2018, 2020). Table 1 shows the experimental and predicted results of IPP production using Novozym 435. The table shows that the values of the residuals are relatively low. This result indicates the normal distribution of error, which further suggests the correlation between the predicted and experimental values. Table 2 shows that the model F-value of 10.45 implies the significance of the model. Furthermore, the high coefficient of determination of $R^2 = 0.9620$ revealed that the statistical relation between the response and selected variables at the confidence of 96.2% and total variation of 3.8% is not addressed by the proposed model. Thus, other minor variables accompanied in the IPA and PA reaction may affect the reaction conversion.

3.3. Regression analysis

The regression analysis of the data sets was performed subsequently to yield corresponding coefficients and create equations that can describe the model. Adequate precision compares the range of the predicted values at the design points to the average prediction error. The value of 10.059 for the current model indicates that the model can be used to navigate the design space. A value of >4 is suitable for desirable models. Meanwhile, the variation coefficient obtained in this context was relatively low (6.89%), which indicated the good precision and reliability of the performed experiments.

"Prob > F″ values of less than 0.0500 and high F-value indicates the significance of the model terms. Table 2 indicates that the Novozym 435 amount variable of "Prob > F″ of <0.0001 is less than that of the IPA/PA molar ratio of 0.0023. In addition, the F-value of Novozym 435 amount variable of 48.63 is higher than that of IPA/PA molar ratio of 16.55. This result indicates that the amount of the Novozym 435 could be considered as a significant variable affecting the conversion value. An empirical equation was generated based on the abovementioned values to estimate the response value and screened uncoded variables at any

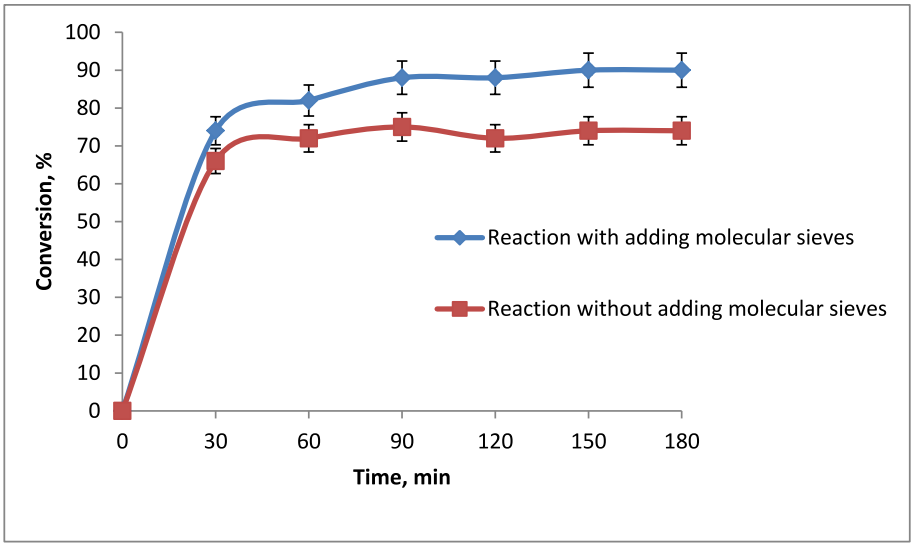

Fig. 1. Effect of the reaction time on the catalyzed production of isopropyl palmitate in a single-phase medium. Temperature: 60°C, Novozym 435: 4% (w/w), and molar ratio of substrate (palmitic acid:isopropyl alcohol of 1:15 and speed of agitation of 150 rpm). The arrow bar indicates the triplicate of the standard deviation.

Table 1
Experimental design of the three variables along with the experimental and predicted values of palmitic acid and isopropyl alcohol conversion (temperature of 60°C, time of 2.5 h, and agitation speed of 150 rpm).

Order	Molar Ratio	Novozym 435%, (w/w)	Molecular Sieves %, (w/w)	Experimental Value	Predicted Value	Residual
1	3	4	1	83.13	79.27	3.86
2	9	2.5	0	82.80	83.60	−0.80
3	9	2.5	5.5	85.50	85.54	−0.037
4	3	1	1	58.00	54.26	4.54
5	9	5	5.5	87.50	86.64	0.86
6	15	4	1	84.66	89.26	−4.60
7	9	2.5	5.5	85.70	85.54	0.16
8	−1	2.5	5.5	50.00	60.01	−10.01
9	3	1	10	67.00	58.78	8.22
10	9	2.5	13	84.48	88.80	−4.32
11	15	1	1	69.54	69.06	0.48
12	9	2.5	5.5	85.90	85.54	0.36
13	9	0	5.5	45.00	50.98	−5.98
14	15	1	10	73.00	73.53	−0.23
15	9	2.5	5.5	85.70	85.54	0.16
16	15	4	10	90.00	90.92	−0.92
17	9	2.5	5.5	85.30	85.54	−0.24
18	9	2.5	5.5	86.00	85.54	0.46
19	3	4	10	84.14	80.99	3.15
20	19	2.5	5.5	85.69	80.81	4.88

Table 2
ANOVA of the enzymatic production of isopropyl palmitate.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	*p*-Value Prob > F
Model	2962.07	9	329.12	10.43	0.0005
A-Molar ratio	522.25	1	522.25	16.55	0.0022
B-Enzyme amount	1534.56	1	1534.56	48.63	<0.0001
C-Molecular sieve	32.71	1	32.71	1.04	0.3324
AB	11.64	1	11.64	0.37	0.5571
AC	1.512E-003	1	1.512E-003	4.793E-005	0.9946
BC	3.93	1	3.93	0.12	0.7314
A∧2	412.36	1	412.36	13.07	0.0047
B∧2	503.89	1	503.89	15.97	0.0025
C∧2	0.80	1	0.80	0.025	0.8769
Residual	315.55	10	31.55		
Corrected total	3277.62	19			

point.

$$Y = +23.21 + 4.103A + 22.19B + 0.567C – 0.141AB – 0.0056AC – 0.113BC – 0.149A^2 – 2.636B^2 + 0.0107C^2 \quad (3)$$

Where Y denotes the conversion, whereas A, B, and C denote the IPA/PA molar ratio, amount of Novozym 435, and percentage of molecular sieves, respectively.

3.4. Response surface plots

3.4.1. Interactive effect of the percentage of molecular sieves and amount of Novozym 435

Fig. 2a represents the variation effect of molecular sieves and amount of Novozym 435 on IPP production at a reaction time of 2.5 h. The plot of the interaction of the response surface yielded optimum values. The figure shows that high response could be achieved at low molecular sieve percentage and high amount of Novozym 435 and at high molecular sieve percentage and high amount of enzyme. Such observation indicates the high significance of the amount of Novozym 435. As shown in Fig. 2a, the conversion obtained at 4% (w/w) of *Candida antarctica* lipase and 1% (w/w) of molecular sieves (at 9:1 isopropanol-to-PA molar ratio) is approximately equal to that obtained at 4% (w/w) of *Candida antarctica* lipase and 10% (w/w) of molecular sieves. Similar findings of the relation between the molecular sieve percentage and amount of immobilized enzyme have been reported by Soo (2003) and Radzi et al. (2011).

3.4.2. Interactive effect of molecular sieves and molar ratio

Fig. 2b indicates the response surface plot as a function of the molar ratio and molecular sieve percentage on IPP synthesis using Novozym 435 at 2.5 h. The interaction response surface plot was generated at a fixed Novozym 435 amount of 2.5% (w/w). The results shown in the figure indicates that conversion increases at any molecular sieve percentage and vice versa, in the proposed range, with the increase of the IPA/PA molar ratio. Molecular sieves of 10% w/w with isopropanol/PA molar ratio of 4:1 favored high conversion of the reaction. Yamaguchi and Mase (1991) and Freitas et al. (2010) have suggested that high molar ratios of alcohols to fatty acids favor the esterification reaction equilibrium toward completion. The last observations are consistent with that obtained in this study.

3.4.3. Interactive effect of the amount of Novozym 435 and molar ratio

The response surface showed in Fig. 2c represents the effect of the amount of Novozym 435 and isopropanol/PA molar ratio on the production of IPP. The response surface plot for the interaction was generated for the optimal values. The figure shows that conversion increases with the increase in the molar ratio and amount of Novozym 435. The isopropanol/PA molar ratio of 15:1 and amount of Novozym 435 (4% w/w) seem to be the favorable parameters for the catalyzed synthesis of IPP (90% of conversion). Conversely, 1% (w/w) of Novozym 435 and 1:1 M ratio resulted in a drastic drop in conversion (less than 60%). The low conversion of 60% was the lowest value recorded in this study, which indicated that the isopropanol/PA molar ratio and amount of Novozym 435 were significant conditions, whereas the percentage of molecular sieves was less significant. The interactive effect is explained using RSM, where "Prob > F″ was <0.0001 for the enzyme amount variable, 0.0023 for isopropanol/PA molar ratio, and 0.3324 for the amount of molecular sieves. Similar findings were reported by Abdelmoez et al. (2016b).

Based on the present findings and RSM, the optimum conditions can be considered as follows: amount of Novozym 435 (4% w/w, 0.4 g), temperature of 60°C, molecular sieve concentration of 10% w/w (1 g), and isopropanol/PA molar ratio of 15:1 (7.78 g IPA + 2.22 PA). The response resulted from the abovementioned conditions was 90%.

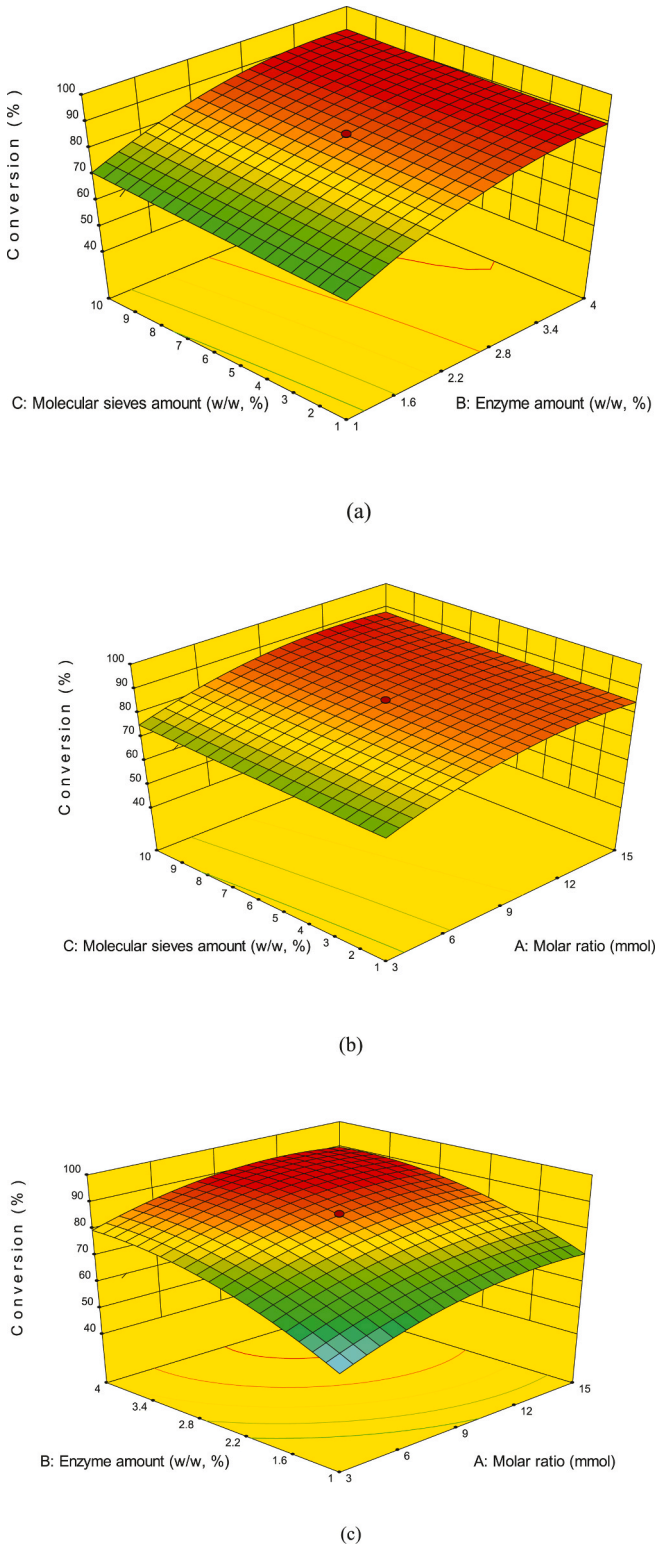

Fig. 2. Response surface plot for the interaction of two parameters affecting isopropyl palmitate synthesis using Novozym 435. (a) Load of molecular sieves versus amount of Novozym 435. (b) Amount of molecular sieves versus molar ratio. (c) Amount of Novozym 435 versus molar ratio.

3.5. Operational stability of Novozym 435

The high cost of commercial enzymes is the main issue hindering the commercialization of the enzymatic process. Although this technology has proven itself in some applications, such as biodiesel and margarine, it has not been applied to the oleochemical industry on a large scale. Therefore, the optimum use of expensive enzymes becomes necessary for an economically feasible process. Lipase reusability is one of the ways that may support the process economy. Many previous papers have tried to reuse lipases through several cycles (Zhong et al., 2013; Keng et al., 2009). In this work, the operational stability of Novozym 435 was evaluated via 15 cycles (Fig. 3). Novozym 435 was separated via filtration after each cycle and then it was removed from the filter paper by immersing it in IPA. Once all immobilized lipases were removed from the filter paper, they were fed to the reactor to start a new batch.

In this study, the operational stability was measured by dividing the conversion of cycle *n* over the conversion of cycle one. This finding indicates that the operational stability with regard to cycle one is equal to 100%. The study of operational stability was performed at 60°C while maintaining the other optimum parameters. This study showed that Novozym 435 maintained more than 95% of its original activity after being used for 15 cycles. Similar results were pointed out for the enzymatic synthesis of 1,3 diacylglycerols in a solvent-free system using lipozyme RM IM by Zhong et al. (2013). Keng et al. (2008) has also reported similar results on lipase-catalyzed palm esters synthesized in a stirred tank reactor using lipozyme RM IM.

With regard to the economic feasibility of ester production using the enzymatic method, a proportional relation between increasing the numbers of enzyme reusability cycles and process economy is found. When the market price of a product is higher than its total manufacturing cost, the process becomes economically feasible. In a previous work, Mustafa et al. (2016b) have investigated the economic viability of glyceryl monolaurate production. The authors suggested that the economic viability becomes valid if the reuse capabilities of the immobilized lipase are improved until 50 cycles. They also showed that the enzyme dose reduced from 2.5 kg/t to only 0.5 kg/t while maintaining 10 cycles of reusability, which will lead to the same result. Hoseny and Mustafa (2020) reported a detailed economic study for 2-ethyl hexyl oleate using Novozym 435 in a fixed bed reactor. The authors suggested that Novozym 435 provided productivity of 2 t/kg. They concluded that their enzymatic process was profitable by 30% with regard to the product market price. In this study, the economic study was beyond the scope. However, the authors performed the same reaction in a fixed bed reactor to investigate the accurate economic viability of IPP using Novozym 435. This data will be presented in the authors' future publications.

3.6. Plant design

A plant design based on a daily capacity of 10 tons was proposed to investigate the process feasibility. PFD performed the semicontinuous

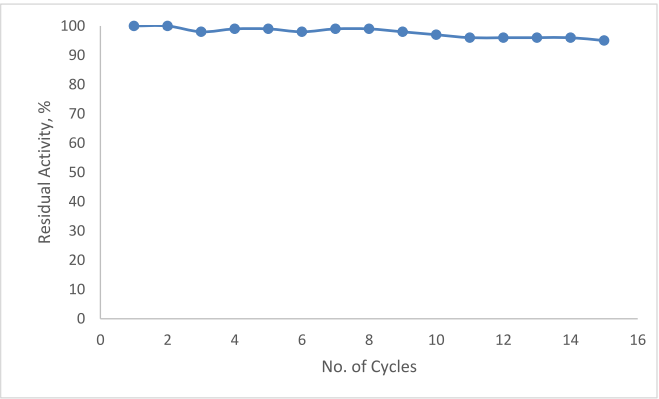

Fig. 3. Operational stability of Novozym 435; reaction conditions for the synthesis of isopropyl palmitate: 4% w/w enzyme amount, 15:1 isopropyl alcohol-to-palmitic-acid molar ratio, and 10% w/w molecular sieve amount at 60°C and 150 rpm.

production of IPP in a solvent-free system. As shown in Fig. 4, PA and IPA were preheated at 60°C in R1 and R2, respectively, and then pumped to the esterification reactor. Pumping was conducted under a controlled flow rate of 92.25 and 324.4 kg/h for fatty acid and alcohols, respectively. The flow rate was calculated on the basis of the obtained optimum alcohol-to-fatty-acid molar ratio. The mixture was then transferred to the main esterification reactor R3. This reactor worked under heating at 60°C and mechanical agitation of 200 rpm for the determined reaction time of 2.5 h. The esterification reaction effectively begun once a 4% w/w of lipases was fed from the top of the reactor through a dosing set DS1 mounted on the top of the reactor R3. Moreover, molecular sieves (MSs), 10% w/w, were fed to the same reactor. After achieving the desired conversion, the slurry mixture was pumped to filter press FP1 by pumping P3 to separate the immobilized enzymes and MS. Thus, the filtrate was a mixture of IPA and PA, whereas the retentate was immobilized enzymes and MS. Avoiding Novozym 435 from denaturation during filtration was important. This could be performed by considering two points: first, the pump P3 was selected from the diaphragm type. During this time, pumping was conducted using air instead of mechanical impellers, which created remarkable shear stresses, thereby affecting the enzymatic activity. Second, controlling the pressure drop of the filter press could be performed by continuously measuring the value of differential pressure (Delta P), which was the difference between the feed pressure inlet and filtrate outlet. The increasing value of the feed pressure indicated the accumulation of retentate. Thus, the minimum pressure difference of 0.5 bar should be maintained to avoid enzyme denaturation.

The enzymes and MS separated previously from FP1 returned to DS1 to catalyze the esterification reaction. They could be gently washed with IPA in the same esterification reactor R3 (between batches) to regenerate Novozym 435 and MS before starting a new batch and then the mixture was sent to FP1 to separate the IPA residue. In this study, the operational stability of MS was equal to the operational stability of Novozym 435. This finding indicates that they are charged to the reactor, reused, and discharged for disposal.

Regarding filter press FP1, retentate squeezing was not made to avoid lipases from shear stresses. Therefore, the clear filtrate of isopropyl palmitate (96.642 kg/h), excess IPA (302.68 kg/h), and traces of PA (10.96 kg/h) was collected into the evaporator EV1. Excess alcohol was separated in EV1 with a mass flow rate of 302.68 kg/h and returned to the alcohol feeding tank. The quality of the returned IPA was measured periodically by checking the moisture content through sampling. When the moisture content increased, the activity of the MSs begun to decrease. At this stage, fresh MSs were added to the reactor. After evaporation, the remaining mixture comprised IPP with a FFA of 10% (as palmitic). The FFA could be removed via neutralization with NaOH solution, which was being injected to the static mixer MS1. The product mixture was then sent to centrifuge CN1 to separate the resulted soap previously formed from the FFA neutralization reaction.

Notably, although the proposed reaction was performed without using a solvent, the medium of the reaction did not require a vigorous mechanical agitation to maintain Novozym 435 suspension because the viscosity of the reactant decreased at the operating temperature of 60°C. Further, the viscosity of the reaction mixture decreased drastically after half an hour of the esterification reaction. This result was primarily due to the high conversion rate of PA and IPA to IPP, which has a lower viscosity than the reactants.

Immobilized enzymes were successfully used in previous works in agitated reactors. One of these works was published by Keng et al. (2008). Keng reported that stirring at 250 rpm is the optimum condition that could keep the lipozyme RMIM particle suspension to produce palm esters. A similar finding was reported by Abdelmoez et al. (2016), who used Novozym 435 to produce glycerin laurate. King could use lipozyme RM IM for 15 cycles without remarkable activity losses. This finding indicates the stable immobilization system for lipozyme RM IM against mechanical stirring and the feasibility of using mechanical agitation with immobilized Novozym 435.

4. Conclusion

In the present work, Novozym 435 was used as a biocatalyst in the esterification reaction of IPA and PA in a solvent-free system. The optimal conditions were as follows: temperature, 60°C; amount of Novozym 435, 4% (w/w); IPA-to-PA molar ratio, 15:1; molecular sieve percentage of 10% (w/w); and time of reaction, 2.5 h. Consequently, a high esterification conversion of 90% was achieved. Furthermore,

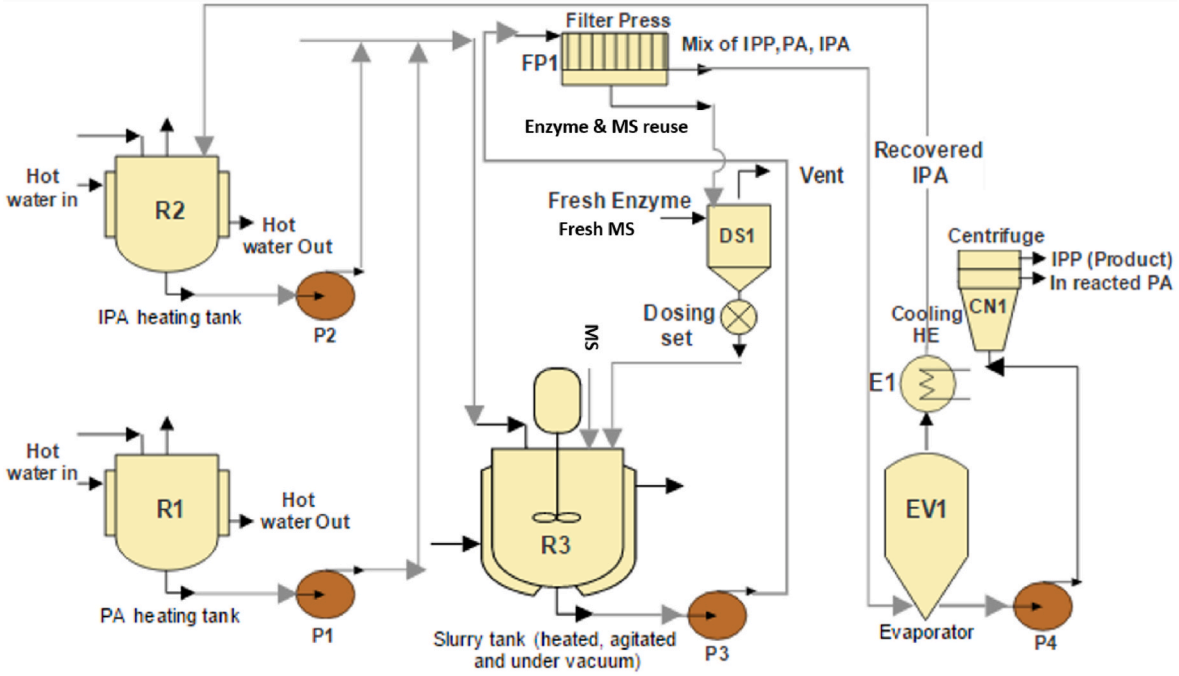

Fig. 4. Semicontinuous production of isopropyl palmitate.

Novozym 435 maintained more than 95% of the original activity after being used for 15 batches. A semicontinuous production line of IPP was further proposed by developing a process flow diagram. Given the environmental benefits of using the enzymatic method, lipases have a higher consumer appeal than those made using chemical methods (particularly in cosmetic and food applications).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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