Research Article

Lipase-Catalyzed Synthesis of Cetyl Palmitate in the Presence of *n*-Hexane and Ethyl Ether

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Cetyl palmitate was produced by the esterification of palmitic acid with cetyl alcohol using *n*-hexane and ethyl ether as solvents and a commercial lipase as a catalyst. The effect of solvents to reagents mass ratio (0.5 : 1–3 : 1), percentage of *n*-hexane in the solvent mixture (0–100%), and reaction temperature (25–55°C) on the reaction rate were evaluated for a fixed amount of enzyme (1 wt% related to the total mass of substrates) in an equimolar mixture of palmitic acid and cetyl alcohol. Temperature and *n*-hexane percentage in the solvent had positive effects on the reaction rate. The total solvent-to-reagent mass ratio showed a negative effect on the reaction rate when a solvent mixture rich in ethyl ether was used. The higher the concentration of *n*-hexane, the lower the effect of the mass ratio of solvents to reagents on the reaction rate. Although the amount of ethyl ether in the solvent mixture had a negative effect on the reaction rate, it had a positive effect on the solubility of the system, that is, the more ethyl ether in the mixture, the lower the solvent to reagents mass ratio required to ensure a homogeneous mixture. A ping-pong bi-bi mechanism-based model was proposed to represent the system kinetics and was well fitted to the experimental data.

1. Introduction

Waxes are long-chain esters derived from fatty acids and alcohols with a chain length of twelve or more carbons [1]. They are used in several industrial applications, such as the production of lubricants, cosmetics, personal care products, pharmaceuticals, wood coatings, antifoaming agents, printing inks, and varnishes [2]. In nature, they are found in the skulls of sperm whales, in the leaves of the carnauba, and mainly in beehives [3]. However, since obtaining wax esters from natural sources is costly and does not meet the current industry demand [4], they are generally synthesized by the esterification of a fatty acid with a long-chain alcohol [5–10]. The most common way to carry out this synthesis is in the presence of an acid catalyst [4]. Despite this approach usually achieving high conversions [6], it has some disadvantages such as problems with corrosion, environmental risks, and especially difficulties in purifying the product [2].

Among the different types of wax esters, cetyl palmitate stands out for its application in the cosmetic industry as an emollient and thickening agent [11]. This wax ester can be synthesized by an enzyme-catalyzed esterification of cetyl alcohol with palmitic acid, in the presence [12] or absence [13] of organic solvents. Although enzymes have a high cost, they can catalyze reactions under mild conditions, avoiding expenses with fuels, steam, and robust reactors [13]. The products also have a higher degree of purity and a lower degree of degradation, so they are easier to purify [14]. The use of a solvent can decrease the viscosity and increase the miscibility of the reaction system while improving the enzyme activity. On the other hand, its use can also pose risks to the environment and increase costs with the subsequent separation and recovery of the solvent [15]. Since both strategies have advantages and disadvantages, defining the best process condition for the synthesis of cetyl palmitate depends on detailed knowledge of how the amount and

composition of a solvent mixture affect the reaction kinetics catalyzed by a given enzyme.

Previous work focused on the esterification of palmitic acid with cetyl alcohol catalyzed by *Lipozyme RM IM* in a solvent-free system [13]. This work covers the synthesis of cetyl palmitate using the same catalyst and *n*-hexane [5, 7, 16] and ethyl ether as solvents. Due to the high melting point of the reagents (49.3–62.9°C), the use of solvents is necessary to work with reaction temperatures below 60°C without precipitation [13]. *n*-hexane was chosen because it is a well-established solvent for use in enzymatic reactions, while ethyl ether was chosen for its greater solubility in the reaction system and low boiling point.

A model based on the ping-pong bi-bi mechanism [12, 17] was proposed to describe the esterification reactions under the investigated conditions. The experimental and modelling data presented in this work can be useful for designing a cost-effective process for enzyme-catalyzed production of cetyl palmitate in the presence of organic solvents.

2. Materials and Methods

2.1. Materials. Palmitic acid (≥98%) and cetyl alcohol (95%) were purchased from Exodus Científica (São Paulo, Brazil) while ethanol (99.8%) and *n*-hexane (99%) were purchased from Neon (São Paulo, Brazil). The commercial enzyme *Lipozyme RM IM* (from *Rhizomucor miehei* immobilized on a macroporous ion exchange resin) was purchased from Sigma-Aldrich (Denmark). Other chemicals were of analytical grade and were used as received.

2.2. Lipase-Catalyzed Esterification. Batch reactions were conducted in a jacketed glass vessel (25 ml) closed with a stopper to avoid solvents and water evaporation. The temperature was set by a circulation thermostatic bath (*VIVO RT4*, with an accuracy of ±0.05°C). Before the set temperature was reached, precise amounts of substrates (molar ratio of palmitic acid to cetyl palmitate of 1 : 1) and solvents (mass ratio between 0.5 : 1 and 3 : 1 in relation to substrates) were added to the reactor. The system was agitated by a magnetic stirrer (*IKA C-MAG HS 4*) at 600 rpm, which was determined in a previous study [12]. After the set temperature was reached, a sample (between 1 and 3 ml) was collected to determine the initial concentration of palmitic acid. The greater the dilution of the substrates in solvents, the greater the sampling volume. After that, the reaction volume was 15 ml, and the reaction was started with the addition of an enzyme (1% related to the mass of substrates). Three reaction parameters were analysed as follows: temperature (25–55°C), solvents to reagents mass ratio (0.5 : 1–3 : 1), and *n*-hexane percentage in the solvent mixture, whose range depended on the mass ratio of solvents to substrates. As a result of the low boiling point of ethyl ether (34.6°C), this last parameter was only evaluated for the reaction temperature of 25°C. For all other temperatures, pure *n*-hexane was used as the solvent. At the end of the reaction, the stirring was interrupted for enzyme decantation, and the

liquid was collected for quantification of remaining free fatty acids, which was used to determine the reaction conversion.

2.3. Determination of Free Fatty Acids. Samples collected at the beginning and at the end of the reaction were titrated in triplicate with 0.1 M NaOH, using alcoholic phenolphthalein solution (95 wt%) as an indicator [13]. The percentage conversion at a reaction time t was calculated by using the following equation:

$$\text{Conversion (\%)} = 100 \, \frac{[PA]_{t=0} - [PA]_t}{[PA]_{t=0}}, \tag{1}$$

where $[PA]_{t=0}$ is the concentration of palmitic acid at the beginning of the reaction and $[PA]_t$ is the concentration of palmitic acid at a time t.

2.4. Determination of Conditions That Guarantee a Miscible System. Before the reaction studies, preliminary experiments were carried out to establish experimental conditions that would guarantee that the reagents would be soluble in the solvents.
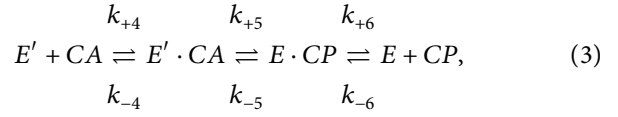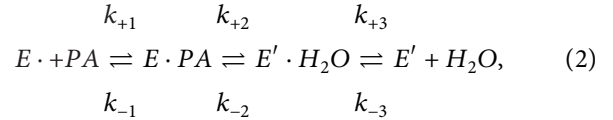
In case the solvent was pure *n*-hexane, a mixture containing a mass ratio of 0.5 : 1 of *n*-hexane to substrates (molar ratio of palmitic acid to cetyl palmitate of 1 : 1) was first heated to 55°C. The temperature was then slowly reduced until a visible solid phase appeared. Then, the temperature was slowly increased until the complete miscibilization of the system was observed. It is important to emphasize that the authors did not consider this last temperature as the phase transition temperature. For this, a more precise methodology would be needed to be employed. This temperature, however, indicates a feasible limit for carrying out the experiment in a miscible system. Once this minimum allowed temperature was determined for a defined *n*-hexane mass ratio in relation to substrates, the amount of *n*-hexane in the system was increased so another temperature was determined. This procedure was repeated to obtain a "*temperature*" vs "*mass ratio of n-hexane to substrates*" curve, above which the reaction system is surely miscible.

In case the solvent was a mixture of *n*-hexane and ethyl ether, the substrates (molar ratio of palmitic acid to cetyl palmitate of 1 : 1) were first heated to 25°C. Ethyl ether was slowly added to the system until complete solubilization was observed. After that, some amount of substrates was added to the system so a solid phase was formed. *n*-hexane was then added to the system until it was miscible again. The addition of *n*-hexane into a system with liquid and solid phases, until a miscible system was obtained, was carried out to establish an adequate value of mass ratio of solvents (with a specific percentage of *n*-hexane) to substrates, above which we can guarantee that we have a miscible system. This procedure (adding substrates to form a solid phase, followed by *n*-hexane addition so that the system is miscible again) was repeated to obtain a "*mass ratio of solvents to substrates*" vs "*percentage of ethyl ether in the solvent*" curve, above which the reaction system is surely miscible at 25°C. This result is important due to the difference in solubility of substrates of

the different solvents used. The greater the amount of n-hexane in the solvent mixture, the greater the mass ratio of solvents to substrate is required to guarantee a miscible system for a constant temperature.

2.5. Enzyme Reuse.

Reaction studies showed that the use of pure ethyl ether as a solvent caused a strong inhibition of enzyme activity. To elucidate whether this inhibition was reversible or irreversible, experiments on reusing the enzyme were conducted. After the reaction was carried out in the presence of ethyl ether, the enzyme was washed and filtered with 40 ml of n-hexane and left in a desiccator for 3 hours. Then, a reaction was carried out using pure n-hexane as solvent.

2.6. Kinetic Modelling.

A reversible ping-pong bi-bi mechanism (equations (2) and (3)) was used to describe the enzymatic esterification.

$$E \cdot + PA \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E \cdot PA \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E' \cdot H_2O \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E' + H_2O, \quad (2)$$

$$E' + CA \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} E' \cdot CA \underset{k_{-5}}{\overset{k_{+5}}{\rightleftharpoons}} E \cdot CP \underset{k_{-6}}{\overset{k_{+6}}{\rightleftharpoons}} E + CP, \quad (3)$$

where E is the free enzyme, PA is palmitic acid, CA is cetyl alcohol, CP is cetyl palmitate, and E' is the substituted enzyme (i.e., with the acyl group). Since the enzyme concentration was much lower than that of the substrates, a steady-state enzyme kinetics (the formation and breakdown of enzyme complexes are equal) was assumed. The reaction rate of palmitic acid (r_{PA}) was then obtained by using the King–Altman method [17, 18] using a free online tool [19] as follows:

$$r_{PA} = \frac{E_0 \left(-n_1 [PA][CA] + n_2 [H_2O][CP] \right)}{\left(\begin{array}{c} d_1 [H_2O][CP] + d_2 [CA][CP] + d_3 [PA][H_2O] + d_4 [PA][CA] \\ + d_5 [CP] + d_6 [H_2O] + d_7 [CA] + d_8 [PA] \end{array} \right)}, \quad (4)$$

where E_0 is the total enzyme concentration.

$$
\begin{aligned}
n_1 &= -k_{-1}k_{-2}k_{-3}k_{-4}k_{-5}k_{-6}, \\
n_2 &= +k_{+1}k_{+2}k_{+3}k_{+4}k_{+5}k_{+6}, \\
d_1 &= k_{-2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-3}k_{-4}k_{-5}k_{-6} + k_{+2}k_{-3}k_{-4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-3}k_{-5}k_{-6} + k_{-1}k_{-2}k_{-3}k_{-4}k_{-6} + k_{-1}k_{-2}k_{-3}k_{+5}k_{-6}, \\
d_2 &= k_{-1}k_{-2}k_{+4}k_{-5}k_{-6} + k_{-1}k_{+3}k_{+4}k_{-5}k_{-6} + k_{+2}k_{+3}k_{+4}k_{-5}k_{-6} + k_{-1}k_{-2}k_{+4}k_{+5}k_{-6} + k_{-1}k_{+3}k_{+4}k_{+5}k_{-6} + k_{+2}k_{+3}k_{+4}k_{+5}k_{-6}, \\
d_3 &= k_{+1}k_{-2}k_{-3}k_{-4}k_{+6} + k_{+1}k_{-2}k_{-3}k_{-4}k_{-5} + k_{+1}k_{-2}k_{-3}k_{+5}k_{+6} + k_{+1}k_{+2}k_{-3}k_{-4}k_{+6} + k_{+1}k_{+2}k_{-3}k_{-4}k_{-5} + k_{+1}k_{+2}k_{-3}k_{+5}k_{+6}, \\
d_4 &= k_{+1}k_{-2}k_{+4}k_{+5}k_{+6} + k_{+1}k_{+3}k_{+4}k_{+5}k_{+6} + k_{+1}k_{+2}k_{+4}k_{+5}k_{+6} + k_{+1}k_{+2}k_{+3}k_{+4}k_{+6} + k_{+1}k_{+2}k_{+3}k_{+4}k_{-5} + k_{+1}k_{+2}k_{+3}k_{+4}k_{+5}, \\
d_5 &= k_{-1}k_{-2}k_{-4}k_{-5}k_{-6} + k_{-1}k_{+3}k_{-4}k_{-5}k_{-6} + k_{+2}k_{+3}k_{-4}k_{-5}k_{-6}, \\
d_6 &= k_{-1}k_{-2}k_{-3}k_{-4}k_{+6} + k_{-1}k_{-2}k_{-3}k_{-4}k_{-5} + k_{-1}k_{-2}k_{-3}k_{+5}k_{+6}, \\
d_7 &= k_{-1}k_{-2}k_{+4}k_{+5}k_{+6} + k_{-1}k_{+3}k_{+4}k_{+5}k_{+6} + k_{+2}k_{+3}k_{+4}k_{+5}k_{+6}, \\
d_8 &= k_{+1}k_{+2}k_{+3}k_{-4}k_{+6} + k_{+1}k_{+2}k_{+3}k_{-4}k_{-5} + k_{+1}k_{+2}k_{+3}k_{+5}k_{+6}.
\end{aligned} \quad (5)
$$

The exact same result was also obtained by Cha [20] when they used the King–Altman method under the steady-state assumption for the ping-pong bi-bi mechanism described by equations (2) and (3) (more information on how equation (4) was obtained by using the King-–Altman online tool [19] can be found in the Supplementary file).

The lumped parameters n_1 and n_2 were written as a function of the reaction temperature according to the Arrhenius equation as follows:

$$
\begin{aligned}
n_1 &= A_1 e^{-E_{a1}/T}, \\
n_2 &= A_2 e^{-E_{a2}/T}.
\end{aligned} \quad (6)
$$

The kinetic model used in this work was defined by the following set of differential equations:

$$\frac{dN_{PA}}{dt} = \frac{dN_{CA}}{dt} = a \cdot r_{PA} \cdot V, \quad (7)$$

$$\frac{dN_{H_2O}}{dt} = \frac{dN_{CP}}{dt} = -a \cdot r_{PA} \cdot V, \quad (8)$$

where a is the relative enzymatic activity, which was considered to be a function of the ethyl ether content in the solvent and was calculated with an empirical expression as follows:

$$a = e^{-k_d [EE]}, \tag{9}$$

where k_d is the deactivation constant and [EE] is the concentration of ethyl ether in the reaction system. The reaction system volume is calculated as follows:

$$V = \sum_{i=1}^{C} N_i \cdot Vm_i, \tag{10}$$

where N_i is the number of moles of component i and Vm_i is the molar volume of component i.

The model parameters were estimated by fitting the model to the experimental data by minimizing the sum of the squared errors between the calculated and experimental conversion values. All simulations and parameter estimation were performed in *Scilab*, the function *code* which was used for solving the initial value problem defined by the set of ordinary differential equations (7) and (8) associated with their initial conditions, while the function *fminsearch*, which uses a Nelder–Mead algorithm, was used to minimize the following objective function:

$$\text{obf} = \sum_{k=1}^{NE} \left(\text{conversion}_k^{\exp} - \text{conversion}_k^{\text{calc}} \right)^2, \tag{11}$$

where *NE* is the number of experimental points used in the parameter estimation. The parameters $A_1, A_2, E_{a1}, E_{a2}, d_1, d_2, d_3, d_4, d_5, d_6, d_7, d_8,$ and kd were the decision variables used during the optimization of equation (11).

3. Results and Discussion

3.1. Kinetic Model. The first results of parameter estimation revealed that the following parameters were not significant (that is, their values were very close to zero after the estimation process): $A_2, d_1, d_2, d_4, d_5, d_6,$ and d_7. Therefore, they were removed from the model. The parameter E_{a2} was also removed since it had no meaning once A_2 was zero. After removing these parameters and dividing equation (4) by d_8, the following expression was obtained:

$$r_{PA} = \frac{-v[PA][CA]}{K[PA][H_2O] + [PA]}, \tag{12}$$

where $v = n_1/d_8$ and $K = d_3/d_8$.

The final kinetic model used in this work was then obtained according to the following system of equations:

$$\frac{dN_{PA}}{dt} = -[E_0] \frac{\left(A_1 e^{-k_d [EE] - E_{a1}/T} \right)[PA][CA]}{K[PA][H_2O] + [PA]} V, \tag{13}$$

$$\frac{dN_{CA}}{dt} = \frac{dN_{PA}}{dt}, \tag{14}$$

$$\frac{dN_{H_2O}}{dt} = -\frac{dN_{PA}}{dt}, \tag{15}$$

$$\frac{dN_{CP}}{dt} = -\frac{dN_{PA}}{dt}. \tag{16}$$

The 4 parameters ($A_1, k_d, E_{a1},$ and K) were estimated by fitting the kinetic model to 22 experimental points and are presented in Table 1. The mean absolute error between the experimental and calculated data was 1.87%. Figure 1 presents a parity plot, where experimental data were compared with those calculated from equations (13)–(16). The points are close to the reference line with errors randomly distributed around zero, indicating that the used model is not biased.

3.2. Influence of n-Hexane Amount and Temperature. Table 2 shows experimental results of the conversion of palmitic acid, after 0.5 h of reaction, as a function of the temperature and the amount of solvent, whose ranges were defined according to the low boiling point of *n*-hexane (68°C) and the region (Figure 2) where the solubility of substrates in the solvent is guaranteed according to the procedure described in Section 2.4.

It was observed that the temperature had a positive effect on the reaction rate, which is in line with that observed in a previous work [13], where it was found that the maximum activity of this enzyme can occur at around 80°C. The amount of solvent, on the other hand, did not affect the reaction rate. This is an interesting result, since the addition of solvent diluted not only the substrates but also the catalyst, which was always added in relation to the substrates. This indicates that there is a positive effect of the dilution of the system on the reaction rate, which compensates the decrease in the concentration of reagents and catalysts. The mentioned effect was contemplated in the mathematical model through the parameter K (Table 1 and (13)). Being *n*-hexane an alkane, it has only hydrophobic interactions with the enzyme, and, although it does not significantly change its global structure and active site, it can affect the enzyme activity by changing the enzyme hydration [21]. Once hydration is crucial for the enzyme activity, it is possible that excess *n*-hexane plays a favourable role in the hydration of the enzyme, therefore compensating the greater dilution for larger amounts of *n*-hexane in the medium.

An important finding of this result is that it is possible to maximize the energy efficiency of the studied reaction by working with the minimum solvent necessary to solubilize

TABLE 1: Estimated parameters of the kinetic model.

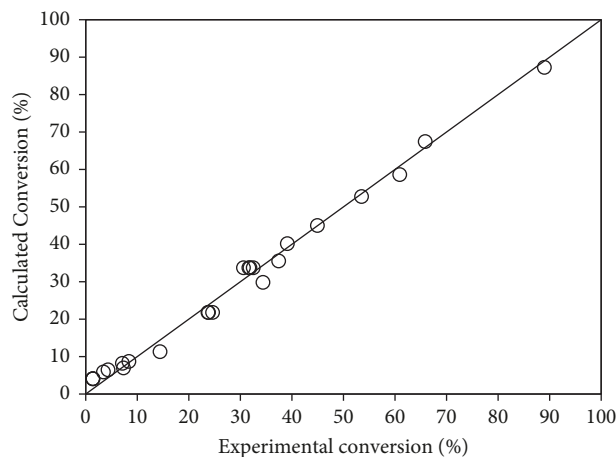| Parameter	Value	Unit
A_1	1.81×10^{15}	$cm^6 \cdot enzyme^{-1} \cdot mol^{-1} h^{-1}$
E_{a1}	5680	K
k_d	353	$cm^3 \cdot mol^{-1}$
K	1.85×10^9	$cm^6 \cdot mol^{-2}$

FIGURE 1: Parity plot comparing experimental and calculated data. Reaction conditions: stirring speed of 600 rpm, initial molar ratio of palmitic acid to cetyl palmitate of 1 : 1, and catalyst loading of 1 wt% (in relation to the total mass of substrates). Reaction temperatures are in the range of 25–55°C, solvent to substrates' mass ratios are in the range of 0.5–3, n-hexane percentages in the solvent mixture (the other solvent is ethyl ether) are in the range of 0–100%, and the reaction times are in the range of 0.5–8 h.

TABLE 2: Effect of temperature and solvent to substrates' mass ratio on the palmitic acid conversion.

Temperature (°C)	n-hexane to reagents mass ratio	Conversion (%)
55	0.5	31.9
	1	32.5
	2	30.6
	3	31.7
37.5	1	23.8
	2	23.7
	3	24.6

Reaction conditions: stirring speed of 600 rpm, initial molar ratio of palmitic acid to cetyl palmitate of 1 : 1, catalyst loading of 1 wt% (in relation to the total mass of substrates), and reaction time of 0.5 h. Standard uncertainty u is u(conversion) = 2.0%.

the substrates, without compromising the reaction rate. Two sets of experiments were also carried out at different temperatures to assess the suitability of the kinetic model to describe the reaction over time, which is confirmed in Figure 3.

3.3. Influence of Solvent Composition. Table 3 shows experimental results of the conversion of palmitic acid, after 0.5 h of reaction, as a function of the amount and compositions of the solvent. The experimental conditions were
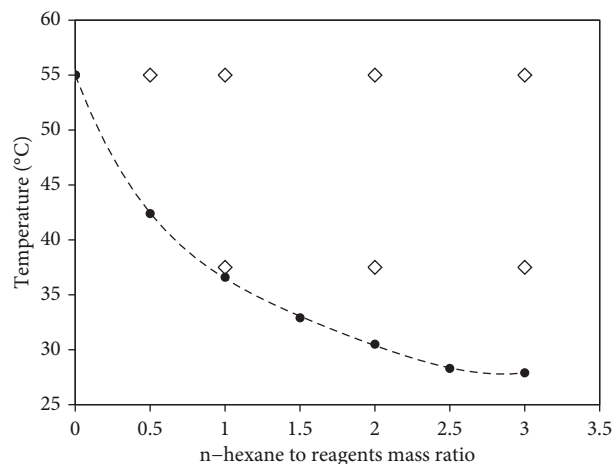

FIGURE 2: Experimental conditions are presented in Table 2 (open lozenges). The region above the dashed line includes the experimental conditions where it is guaranteed that the reagents (molar ratio of palmitic acid to cetyl palmitate of 1 : 1) are completely soluble in the solvent.
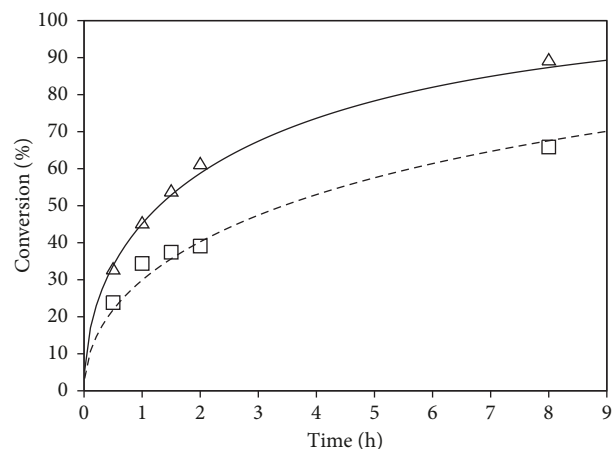

FIGURE 3: Effect of temperature on the esterification of palmitic acid with cetyl alcohol using n-hexane as solvent. (□ – –) 37.5°C; (△ —) 55°C. Reaction conditions: stirring speed of 600 rpm, initial molar ratio of cetyl alcohol to palmitic acid of 1 : 1, catalyst loading of 1 wt% (in relation to the mass of substrates), and n-hexane to substrates' mass ratio of 1 : 1.

determined to ensure that the reagents were completely solubilized in the solvent (Figure 4) according to the procedure described in Section 2.4. Studies involving ethyl ether were conducted only at 25°C because of its low boiling point (34.6°C). Unlike what was observed for pure n-hexane, the reaction rate is negatively affected by the mass ratio of solvents to reagents when a solvent mixture rich in ethyl ether is used.

Figure 4 shows that the greater the fraction of ethyl ether in the solvent mixture, the greater the solubility of the substrates. On the other hand, the greater the activity of the enzyme, the higher the concentration of n-hexane in the solvent (Table 3). Ethyl ether is more hydrophilic than n-hexane, so it can have a greater tendency to strip the essential

TABLE 3: Effect of amount and composition of the solvent on the palmitic acid conversion.

Solvent (ethyl ether + n-hexane) to reagents mass ratio	n-Hexane percentage in the solvent (wt%)	Conversion (%)
	0	1.4
3	25	3.4
	50	7.1
	75	14.4
2	25	4.3
	50	8.4
1.5	25	7.3

Reaction conditions: stirring speed of 600 rpm, initial molar ratio of palmitic acid to cetyl palmitate of 1 : 1, catalyst loading of 1 wt% (in relation to the total mass of substrates), reaction time of 0.5 h, and temperature of 25°C. Standard uncertainty u is u(conversion) = 2.0%.
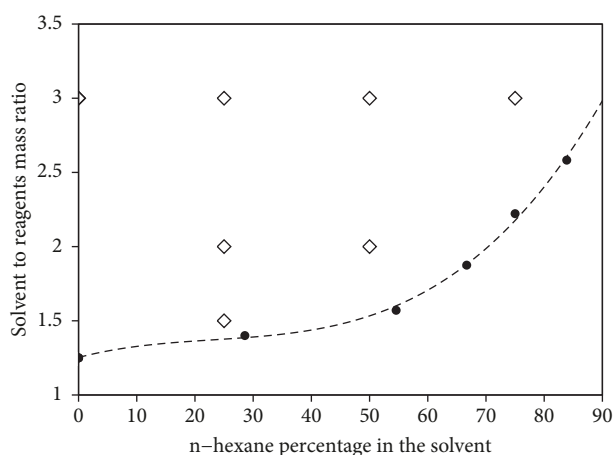

FIGURE 4: Experimental conditions are presented in Table 3 (open lozenges). The region above the dashed line includes the experimental conditions where it is guaranteed that the reagents (molar ratio of palmitic acid to cetyl palmitate of 1 : 1) are completely soluble in the solvent mixture at 25°C.

TABLE 4: Experimental conditions used in the study for the reuse of the enzyme.

Condition	1	2
n-Hexane percentage (wt%)	0	100
Temperature (°C)	25	55
Solvent (ethyl ether + n-hexane) to reagents' mass ratio	3	
Stirring speed (rpm)	600	
Catalyst loading (wt%)	1	
Reaction time (h)	0.5	
Conversion (wt%)	1.5	27.9

4. Conclusions

Commercial immobilized lipase *Lipozyme RM IM* was shown to be effective for cetyl palmitate wax ester production by esterification in the presence of n-hexane. It was found that the n-hexane (reactants + enzyme) mass ratio did not significantly affect the reaction rate. The reaction rate was positively affected by temperature, whose dependence was described by an Arrhenius equation.

The presence of ethyl ether in the solvent mixture allows working at lower reaction temperatures with smaller total amounts of solvent. However, the greater its fraction, the lower the enzymatic activity. It was also found that the loss of activity, attributed to the ethyl ether present in the system, was reversible.

The proposed kinetic model, which contains only 4 adjustable parameters, was capable of representing the reaction system well.

layer of water around the enzyme, which is essential for catalytic activity, leading to a decrease in the enzyme activity [21].

These results reveal that the definition of an optimal solvent composition for the studied reaction is not obvious when the process as a whole is considered. A solvent richer in n-hexane allows higher reaction rates but requires larger reaction systems with larger amounts of solvent, which should affect fixed and variable costs associated with the construction of reactors and with energy expenditure in the purification step.

Since the use of pure ethyl ether showed a strong negative effect on enzyme activity, an enzyme reuse experiment was carried out to verify whether this effect was permanent or only during the exposure of the enzyme to the solvent. First, a reaction was conducted using pure ethyl ether as a solvent (see condition 1 of Table 4). Then, the enzyme was washed and used in a reaction with pure n-hexane as a solvent (see condition 2 in Table 4), resulting in a conversion of 27.9 wt%. This value is slightly smaller than the obtained at the same conditions using a fresh enzyme (31.7 wt%), indicating that the exposure of the enzyme to ethyl ether decreases its activity but does not cause a reversible inhibition.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Supplementary Materials

The details of how equation (4) was obtained by using the King–Altman online tool [19] are given in the Supplementary file. (*Supplementary Materials*)

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