A simplified kinetic model to describe the solvent-free enzymatic synthesis of wax esters

Short title: *Kinetic model of the solvent-free enzymatic synthesis of wax esters*

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ABSTRACT

BACKGROUND: The use of biotechnological processes at industrial scale is a promising tool to replace conventional synthesis as an efficient and eco-friendly technology. For that purpose, the kinetic modelling of an in-lab optimized enzymatic process prior scaling-up is of great utility.

RESULTS: In this work, a kinetic model for the solvent-free synthesis of cetyl laurate, myristate palmitate and stearate using different commercial immobilised lipases has been developed. In order to describe the esterification process of the cetyl esters separately and as a mixture similar to natural spermaceti, a pseudo-first order kinetic has been proposed and tested. A relation between the inverse values of the kinetic constant and the amount of biocatalyst has been observed. The effect of temperature on the reaction rate can be accurately described by Arrhenius equation except for immobilised *Thermomyces lanuginosus*, which appears to be less resistant to temperatures above 70 ºC.

CONCLUSION: Low deviations between experimental and predicted values ($R^2 \geq 0.99$) point out that this pseudo-first order kinetic model can be considered valid for the data range studied. In addition, spermaceti's reaction rate can be successfully predicted through a weighted average of the kinetic constants obtained during the synthesis of each cetyl ester. This simple but accurate kinetic model for describing the solvent-free enzymatic biosynthesis of wax esters from spermaceti may contribute to wide spreading the application of lipases as industrial catalysts.

KEYWORDS: Kinetics, modelling, lipases, biocatalysis.

NOMENCLATURE

A	acid
B	alcohol
P	ester
$(r)_{V_R}$	mass transfer rate of acid (mol L^{-1} min^{-1})
$k_L a$	mass transfer volumetric coefficient (min^{-1})
C_A	acid concentration in the fluid (mol L^{-1})
C_A^*	acid concentration at enzyme's vicinity (mol L^{-1})
K_A	adsorption equilibrium constant (L mol^{-1})
C_{Al}	acid concentration adsorbed on the active site (mol g^{-1})
C_l	concentration of available enzyme active sites related to the mass of biocatalyst used (mol g^{-1})
L	total concentration of active sites related to the mass of biocatalyst used (mol g^{-1})
$(r)_{m_E}$	reaction rate referred to the biocatalyst mass (mol g^{-1} min^{-1})
k	kinetic constant of the enzymatic reaction (min^{-1})
C_E	biocatalyst concentration in the reaction medium (g L^{-1})
m_E	mass of biocatalyst in the reactor (g)
V_R	reactor volume (L)

1	X	conversion
2	k_r	Pseudo-first order kinetic constant (min^{-1})
3	C_{A0}	initial concentration of acid (mol L^{-1})
4	t	reaction time (min)
5	k_0	Arrhenius pre-exponential factor (min^{-1})
6	E_a	activation energy (J mol^{-1})
7	R	ideal gas constant (Pa m^3 mol^{-1} K^{-1}),
8	T	temperature (K)
9		

INTRODUCTION

Although the term Green Chemistry had been used for many years, it was not until 1998 when it got formal recognition with the publication of the Green Chemistry 12 Principles.[1] Biocatalytic processes completely fulfil with most of these postulates:

- Enzymes are catalysts produced from renewable sources and are biodegradable.
- Enzyme's regio- and enantio- selectivity promotes atom economy, reduces derivatives (blocking and protecting groups, etc.) and waste production.
- The possibility of using mild reaction conditions in terms of temperature, pressure, aggressive chemicals or pH leads to energy-efficient and safer synthetic pathways, and thus, to potentially safer industrial processes.
- If aqueous medium or solvent-free conditions are used and product yield is high enough, organic solvents or post-treatment auxiliary substances can be avoided.

One of the most popular enzymes in industrial synthesis are immobilised lipases, as they have a wide range of application and can be easily handled and reused, reducing process economic cost.[2] Lipases can be used to produce a wide variety of esters with many applications:[3–5] lubricants, food additives, coating materials, etc. Wax esters are those with a chain length of 12 carbons of more, among them, esters derived from cetyl alcohol stand out because of their emollient characteristics.[6,7] In fact, natural spermaceti, a mixture of cetyl esters from C12 to C18 fatty-acids traditionally extracted from sperm whales, has probably been one of the most used cosmetic ingredients in cold creams and ointments formulation.[8–10]

Due to their commercial interest, the lipase-based production of such esters has been object of a great number of publications dealing with process optimisation at laboratory scale.[11–14] Those studying the kinetics of the enzymatic process are particularly

interesting for its future scaling up and proper cost estimation on an industrial level. Particularly, the work focused on the kinetic modelling of the esterification reaction to produce wax esters under solvent-free conditions, has been approached from different assumptions: some do not consider diffusional limitations,[15] while others include a semi-empirical term that take into account mass transfer limitations.[16] In any case, the resulting models are complex and rely on a huge number of parameters to describe how conversion varies with time, which makes even more laborious process simulation and reactor design.

In order to ease this task, this work proposes a simplified mathematic model which describes the solvent-free enzymatic synthesis of the commonly used spermaceti cetyl esters separately and as a mixture and its validation with several commercial immobilised lipases.

MATERIAL AND METHODS

Experimental data

Experimental data for the modelling of the enzymatic synthesis of 20 g of cetyl laurate, myristate, palmitate and stearate or the spermaceti analogue in a solvent-free medium is described by the authors in previous work.[17,18] The process was performed in a jacketed Parr 5101 series tank reactor, equipped with an overhead four-bladed impeller, under low pressure and inert N_2 atmosphere conditions. Four commercial immobilised lipases were used:

- CalB immo Plus, made of *Candida antarctica* lipase B (CalB) adsorbed in DVB/methacrylate resin ECR1030M.

- Novozym® 435, CalB immobilised by adsorption on Lewatit® VP OC 1600 carrier.
- Lipozyme® RM IM, which is based on *Rhizomucor miehei* lipase (Rml) immobilised on the phenol-formaldehyde copolymers carrier Duolite ES 562.
- Lipozyme® TL IM, a lipase from *Thermomyces lanuginosus* (Tll) immobilised on a cationic silicate.

In particular, the results from biocatalyst concentration and process temperature optimisation[17,18] were the one used to validate the kinetic modelling proposed for the lipase-based esterification process. Experimental conversion (X) was calculated based on acid value measurements.[19]

Software

The fitting of the experimental data to the kinetic model was performed by using the software CurveExpert Basic 1.40 by creating a custom regression model. The software allowed to easily and quickly determining the kinetic constant with its correlation coefficient and standard error.

KINETIC MODEL

Model hypothesis

The biocatalytic process takes place through the following consecutive steps:

1. Diffusion of the acid (*A*) and the alcohol (*B*) from the reaction bulk to the surface of the carrier.
2. Adsorption of *A* on the lipase active site.

3. Formation of an acyl-enzyme intermediate complex by covalent binding between *A* and the active site serine residue. Release of water.
4. Nucleophile attack of the alcohol to produce the cetyl ester (*P*).
5. Desorption of *P*.
6. Diffusion of *P* from the surface of the biocatalyst to the reaction bulk.

Considering that the substrates are consumed only on the enzymatic surface, it can be assumed that the substrates concentrations at biocatalyst surrounding area, C_A^* and C_B^*, are much lower than the ones in the bulk reaction, C_A and C_B. Besides, as all the reactions were performed with a molar ratio of 1:1 (stoichiometric), substrates concentrations must be equal during the whole process.

Taking into account the above mentioned considerations, the kinetic model has been developed as follows.

Model equations

The mass transfer rate of acid, *A*, from the bulk reaction medium to the surface of catalytic particles, referred to the volume of the reactor, V_R, can be written by following the equation:

$$(r)_{V_R} = k_L a\left(C_A - C_A^*\right) \qquad \text{(equation 1)}$$

where: $(r)_{V_R}$ is the mass transfer rate of acid (mol L^{-1} min^{-1}),

$k_L a$ is the mass transfer volumetric coefficient (min^{-1}),

C_A is the acid concentration in the reaction medium (mol L^{-1}),

C_A^* is the acid concentration at enzyme's vicinity (mol L^{-1}).

In addition, it can be assumed that the process of adsorption of A on the active site of the enzymes, l, reaches the equilibrium:

$$A^* + l \leftrightarrow Al \qquad \text{(equation 2)}$$

whose adsorption equilibrium constant can be defined as:

$$K_A = \frac{C_{Al}}{C_A^* C_l} \qquad \text{(equation 3)}$$

where: K_A is the adsorption equilibrium constant (L mol^{-1}),

C_{Al} is the acid concentration adsorbed on the active sites (mol g^{-1}),

C_A^* is the acid concentration at enzyme's vicinity (mol L^{-1}),

C_l is the concentration of free active sites of enzyme (mol g^{-1}).

The balance of active sites establishes that the total concentration of active sites related to the mass of biocatalyst used, L (mol g^{-1}), is given by:

$$L = C_{Al} + C_l \qquad \text{(equation 4)}$$

Thus, from equation 3:

$$C_l = \frac{C_{Al}}{K_A C_A^*} \qquad \text{(equation 5)}$$

and by substituting equation 5 in equation 4:

$$L = C_{Al}\left(1 + \frac{1}{K_A C_A^*}\right) = C_{Al}\left(\frac{1 + K_A C_A^*}{K_A C_A^*}\right) \qquad \text{(equation 6)}$$

and:

$$C_{Al} = \left(\frac{K_A L C_A^*}{1 + K_A C_A^*} \right) \qquad \text{(equation 7)}$$

On the other hand, since the reaction rate should be proportional to the concentration of acid adsorbed on the biocatalyst, C_{Al}, and taking into account equation 7, the enzymatic reaction rate referred to the biocatalyst mass, $(r)_{m_E}$ (mol g^{-1} min^{-1}), can be expressed as:

$$(r)_{m_E} = kC_{Al} = \frac{kK_A L C_A^*}{1 + K_A C_A^*} \qquad \text{(equation 8)}$$

where k is the kinetic constant of the enzymatic reaction (min^{-1}).

As previously mentioned, C_A^* should be very small, so equation 8 can be simplified to:

$$(r)_{m_E} = kC_{Al} = kK_A L C_A^* \qquad \text{(equation 9)}$$

Since mass transport and the enzymatic reaction take place in series, rates of these two steps should be equal, as long as both are referred either to the reactor volume or to the amount of biocatalyst in reaction medium. Thus:

$$k_L a\left(C_A - C_A^*\right) = C_E k K_A L C_A^* \qquad \text{(equation 10)}$$

where C_E is the biocatalyst concentration (g L^{-1}).

From equation 10 the concentration at the surroundings of the enzyme can be obtained:

$$C_A^* = \left(\frac{k_L a}{k_L a + k K_A L C_E} \right) C_A \qquad \text{(equation 11)}$$

By substituting C_A^* in equation 9, the reaction rate can be expressed as follows:

$$(r)_{m_E} = \left(\frac{k_L a k K_A L}{k_L a + k K_A L C_E}\right) C_A \qquad \text{(equation 12)}$$

If m_E (g) is the enzyme amount in the reactor and V_R the reactor volume (L), the enzyme concentration, C_E, is given by:

$$C_E = \frac{m_E}{V_R} \qquad \text{(equation 13)}$$

According to equation 13, equation 12 can be rewritten as:

$$(r)_{m_E} = \left(\frac{k_L a k K_A L V_R}{k_L a V_R + k K_A L m_E}\right) C_A \qquad \text{(equation 14)}$$

Considering the acid mass balance in the batch reactor:

$$V_R \frac{dC_A}{dt} + m_E (r)_{m_E} = 0 \qquad \text{(equation 15)}$$

by substituting equation 14 in equation 15, and dividing by V_R, the following expression can be obtained:

$$\frac{dC_A}{dt} + \left(\frac{k_L a k K_A L m_E}{k_L a V_R + k K_A L m_E}\right) C_A = 0 \qquad \text{(equation 16)}$$

Therefore, a pseudo-first order kinetic constant, k_r, can be defined as:

$$k_r = \frac{k_L a k K_A L m_E}{k_L a V_R + k K_A L m_E} \qquad \text{(equation 17)}$$

and by substituting equation 17 in equation 16, the equation which describes acid mass balance in a batch reactor considering a first order kinetics can be expressed as:

$$\frac{dC_A}{dt} = -k_r C_A \qquad \text{(equation 18)}$$

From the integration of equation 18 with the initial condition shown in equation 19, the variation of C_A along the time is obtained (equation 20):

$$t = 0;\ C_A = C_{A_0} \qquad \text{(equation 19)}$$

$$C_A = C_{A0} e^{-k_r t} \qquad \text{(equation 20)}$$

where: C_{A0} is the initial concentration of acid (mol L^{-1})

t is reaction time (min).

By defining the conversion, X, of A as:

$$X = \frac{C_{A_0} - C_A}{C_{A_0}} \qquad \text{(equation 21)}$$

from equations 20 and 21, the expression for the variation of conversion with time is deduced:

$$X = 1 - e^{-k_r t} \qquad \text{(equation 22)}$$

Consequently, from the fitting of the experimental data to equation 22, it is possible to determine the value of the pseudo-first order kinetic constant, k_r, for the enzymatic synthesis of the spermaceti esters, when produced separately or as a mixture.

Finally, the relation of k_r with the amount of biocatalyst in the reaction medium, $m_{E,}$ can be deduced from equation 17 as follows:

$$\frac{1}{k_r} = \frac{1}{k_L a} + \frac{V_R}{k K_A L} \frac{1}{m_E} \qquad \text{(equation 23)}$$

Thus, the validity of the proposed model can be checked not only by fitting the experimental data of conversion to equation 22, but also by fitting the values obtained for k_r to equation 23, as described in the Result and Discussion section.

RESULTS AND DISCUSSION

Model fitting to the synthesis of the spermaceti cetyl esters separately.

The kinetic model previously detailed has been used to describe the enzymatic synthesis of the four main components of spermaceti. For this purpose, the experimental conversion data obtained during the synthesis of the four cetyl esters by varying biocatalyst concentration,[17,18] have been fitted to equation 22 using the software CurveExpert Basic 1.40. The results attained are shown in Figures 1 to 4, where the solid lines represent the calculated conversions and the dots the experimental ones.

Figures 1-4 show that the pseudo-first order model proposed accurately predicts the behaviour of the system from a kinetic point of view. Particularly, the model predicts the results with a good level of precision in the case of the processes catalysed by CalB immo Plus, Novozym® 435 and Lipozyme® RM IM. In the case of Tll immobilised derivative, it can also be observed a good correlation between experimental and calculated data, except when the lowest amounts of Lipozyme® TL IM were assayed. This may be explained by the fact that, in this last case, the reaction step may have more influence due to the lower activity of the biocatalyst, which justifies that the optimum amount selected for Lipozyme® TL IM during the experimental optimization was twice the quantity chosen for the other immobilised enzymes.[17,18]

(FIGURE 1)

(FIGURE 2)

(FIGURE 3)

(FIGURE 4)

Additionally, the model can be easily checked by comparing the values of the calculated and the experimental conversions. As it can be observed in Figure 5, an excellent degree of agreement has been obtained, with high determination coefficients, R^2.

(FIGURE 5)

Besides, Table 1 gathers the values of the kinetic constant, k_r, for all the experiments, which have been calculated with high correlation coefficients by using equation 22. Comparing the results obtained when the same amount of each biocatalyst is used, it can be noticed that the highest (and similar) constants are achieved for CalB biocatalysts, while the lowest values are obtained for Tll derivative. This might be due to the higher hydrophobicity of CalB immo Plus and Novozym® 435 methacrylate matrix compared to the ionic exchange carriers used to produce the Lipozyme biocatalysts.[20]

From Table 1, it can also be detected a slight influence of the carbon chain of the product synthesised on the pseudo-first order kinetic constant. In general, its value is lower for CS than for CL, but these differences are so small that in the practice the acid chain length do not significantly affects reaction rate for the esters under study.

Finally, from Table 1 it can be concluded that the value of the pseudo-first order constant increases as the amount of immobilised derivative does. However, this augmentation of k_r does not appear to be directly proportional to m_E, as it is particularly evidenced for the highest quantities of biocatalyst used. In this sense, equation 23 of the

kinetic model proposes that there could be a linear relationship between the inverses of these variables:

$$\frac{1}{k_r} = \frac{1}{k_L a} + \frac{V_R}{kK_A L}\frac{1}{m_E} \qquad \text{(equation 23)}$$

Table 2 shows the results of fitting of $1/k_r$ (represented by y) vs. $1/m_E$ (x) and its corresponding determination coefficient (R^2), whose values over 0.99 even for Lipozyme® TL IM, confirm the validity of the kinetic model. In the specific case of the Tll biocatalyst, it is also noticeable the high values for the slope of the fitting, compared to the others commercial derivatives. According to equation 23, the mathematical expression of the slope contains in its denominator the kinetic constant of the enzymatic reaction (k) and the total number of active centres (L) that might be lower for this biocatalyst and thus, confirms the hypothesis that the reaction step may have more importance for the Tll catalysed esterification process.

(TABLE 1)

(TABLE 2)

Model fitting to one-step synthesis of spermaceti

In the subsequent sections, the pseudo-first order kinetic model will be tested on the biocatalytic synthesis of the spermaceti analogue under solvent-free conditions.

Effect of the temperature

In Figure 6 the experimental conversion measured during the one-step process performed at different temperatures [17,18] are compared to the calculated values by using the kinetic model. These results confirm the ability of the kinetic model to predict how

the one-step solvent-free enzymatic process will evolve, although less precisely for Lipozyme® TL IM. In the same figure, it can also be noticed the negative effect of increasing temperature from 70 to 80 ºC on Tll biocatalyst, which has been reported by several authors during the synthesis of various esters.[21–23]

(FIGURE 6)

On the other hand, the effect of temperature on the kinetic constant can be easily studied through the Arrhenius equation:

$$\ln k_r = \ln k_0 - \frac{E_a}{RT} \qquad \text{(equation 24)}$$

where: k_0 is the pre-exponential factor (min^{-1}),

E_a is the activation energy ($J\ mol^{-1}$),

R is the ideal gas constant ($Pa\ m^3\ mol^{-1}\ K^{-1}$),

T is the temperature (K).

Table 3 shows the values of k_r calculated by the pseudo-first order model and their dependence with temperature through equation 24. As expected, the kinetic constant values increase with temperature except for Lipozyme® TL IM, which, as previously observed in Figure 6, appears to be subject of thermal deactivation. Analysing the determination coefficients, it is obvious that the relation between these variables is accurately described by the Arrhenius equation for the remaining biocatalysts.

In addition, the same trends regarding the performance of the different commercial lipases are observed for the one-step synthesis of spermaceti, i.e., for a fixed temperature, the CalB derivatives exhibit the greatest activity, followed by Rml and then by Tll biocatalyst, despite the fact that in this last case, the amount of immobilised

enzyme is used was two times higher (1 g vs 0.5 g for CalB immo Plus, Novozym® 435 and Lipozyme® RM IM).

(TABLE 3)

Prediction of the kinetic constant for the one-step synthesis of spermaceti.

Since natural spermaceti is a mixture of cetyl laurate, myristate, palmitate and stearate in a specific proportion (that might slightly vary due to its animal origin),[8,9,24] it is interesting to determine if there is any relation between the value of the kinetic constant for the simultaneous synthesis of the spermaceti and the ones obtained for each cetyl ester, taking into account their importance in the composition of the spermaceti.

For this purpose, new values of k_r for the biocatalytic analogue were calculated as the weighted average of the pseudo-first order constants attained for each ester separately, which are compared in Table 4 to the values obtained during the fitting of the experimental data. As it can be noticed, weighted values of the kinetic constants are quite similar to the ones returned by the model fitting to the one-step synthesis, even for the cases where differences between the cetyl esters kinetic constants are more significant.

(TABLE 4)

CONCLUSIONS

This work proves the possibility of modelling the kinetics of a solvent-free enzymatic process to obtain the main components of the spermaceti through a simplified

mathematical approach. In this sense, the esterification catalysed through commercial immobilised lipases accurately fits a pseudo-first order model, where the rate coefficient of the process is chiefly dependent on biocatalyst amount and temperature, but the influence of the carbon chain length is negligible. In addition, it has been observed that process kinetic constant for the synthesis of spermaceti can be easily calculated by means of the weighted average of the constant values for each cetyl ester separately. Thus, this model avoids the need on harsh determination of kinetic parameters with a good predictive capacity, and so, eases reactor design for future scaling-up.

FIGURES

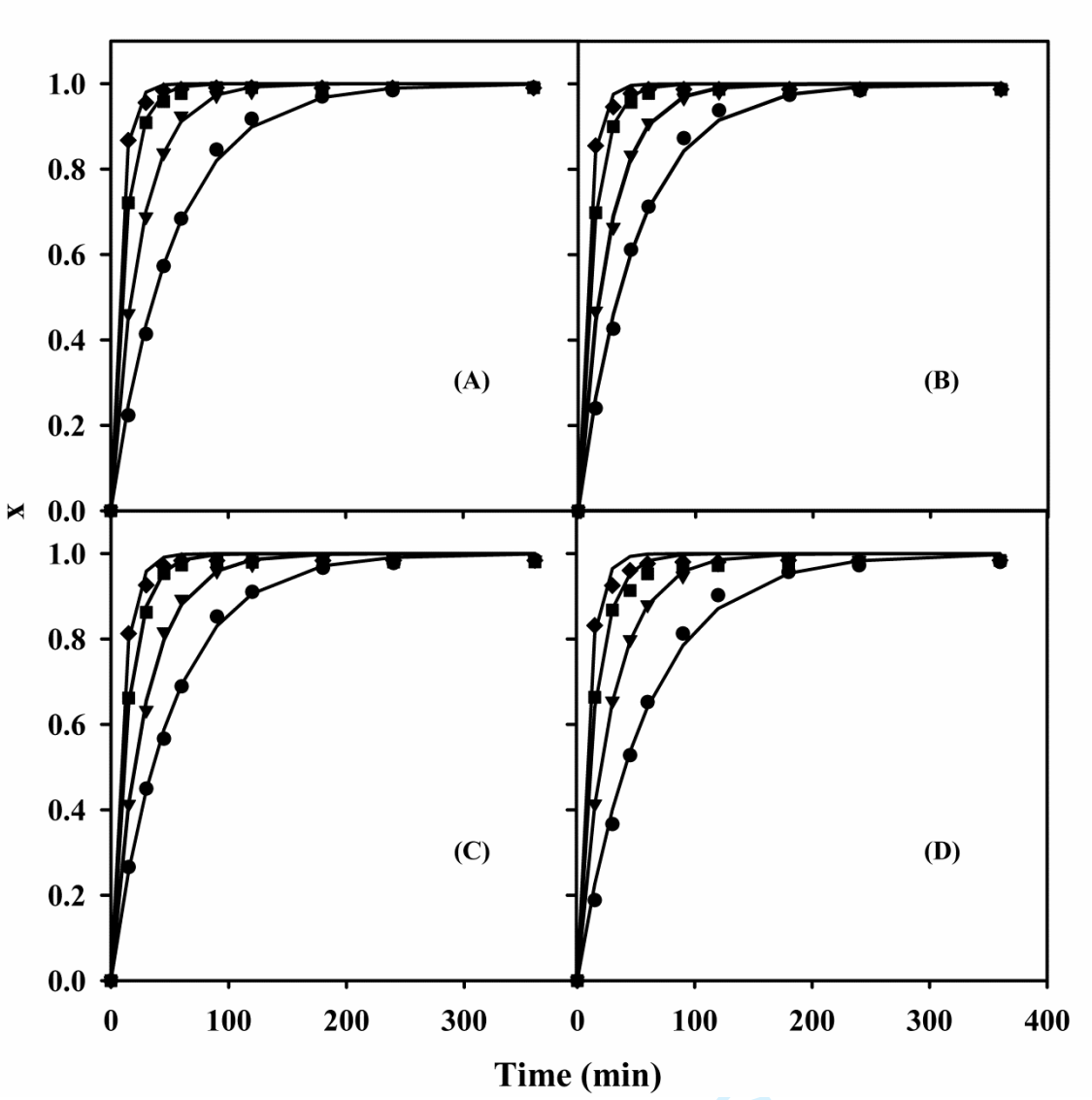

Figure 1. Kinetic model fitting to the synthesis of (A) cetyl laurate, (B) cetyl myristate, (C) cetyl palmitate and (D) cetyl stearate with CalB immo Plus: (●) 0.125, (▼) 0.250, (■) 0.500, (♦) 1.000 g and (-) model. Experimental conditions: 20 g of substrates, 1:1 molar ratio, 70ºC, 350 rpm, 54 L/h N_2 and 21.3 kPa.

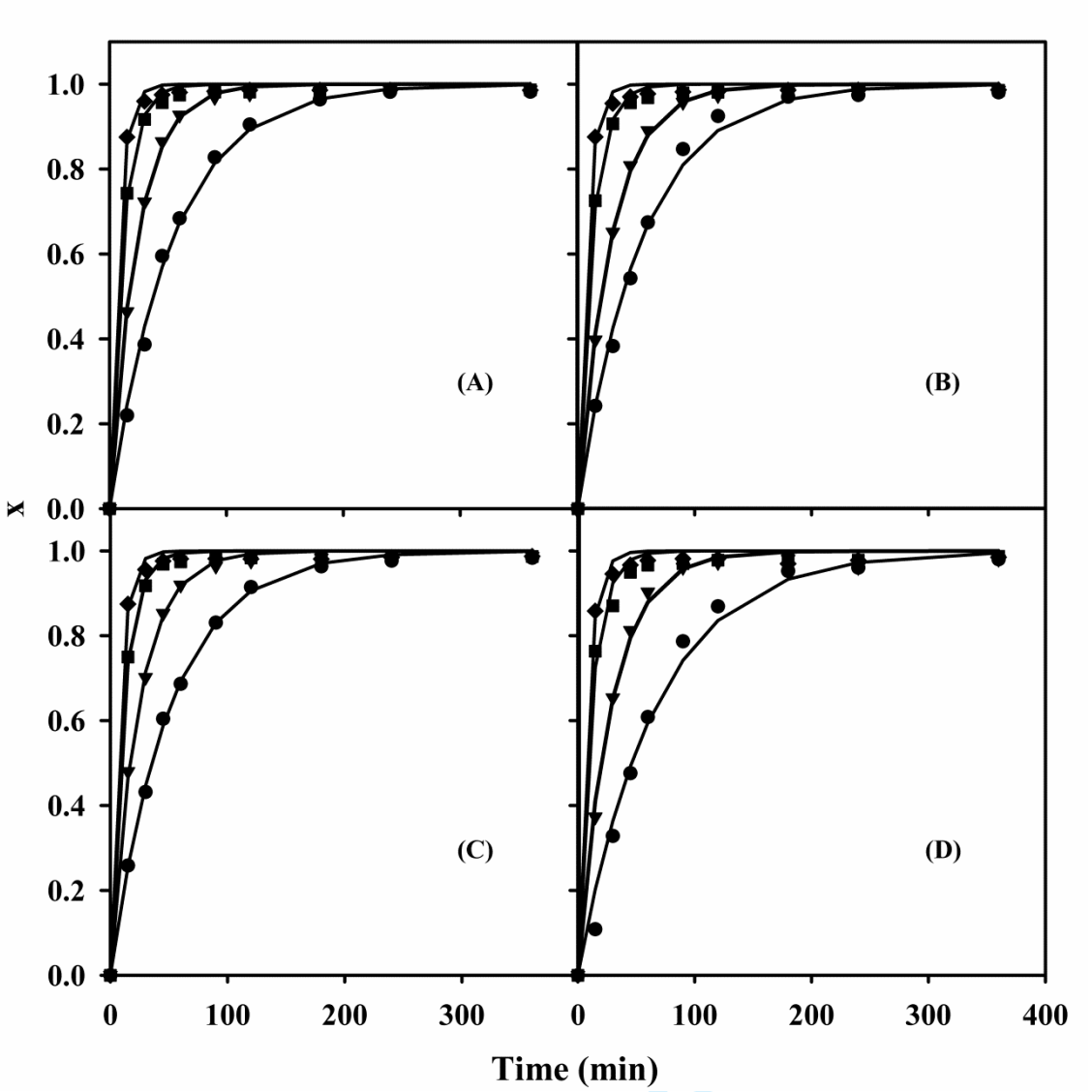

Figure 2. Kinetic model fitting to the synthesis of (A) cetyl laurate, (B) cetyl myristate, (C) cetyl palmitate and (D) cetyl stearate with Novozym® 435: (●) 0.125, (▼) 0.250, (■) 0.500, (♦) 1.000 g and (-) model. Experimental conditions: 20 g of substrates, 1:1 molar ratio, 70ºC, 350 rpm, 54 L/h N_2 and 21.3 kPa.

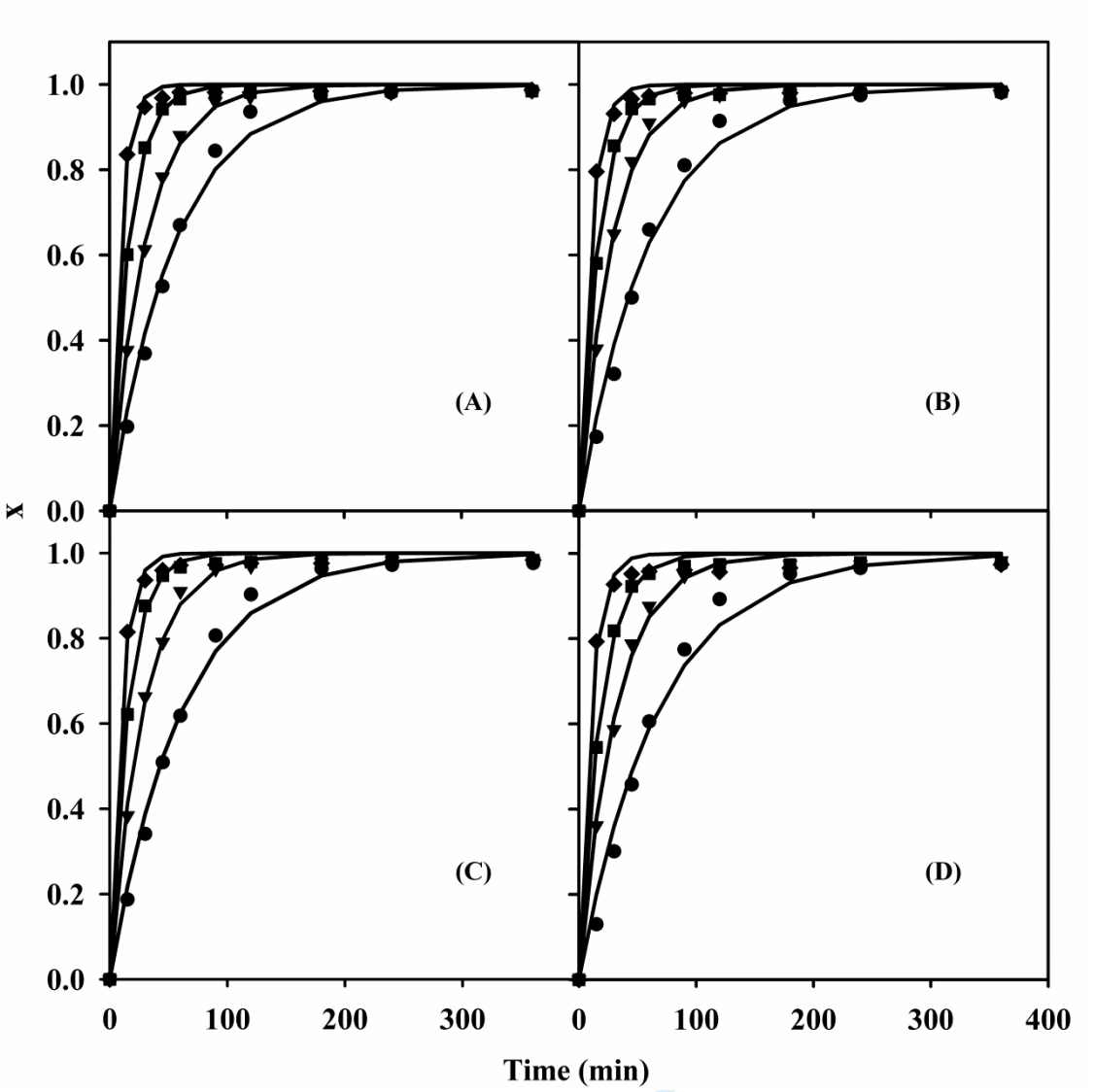

Figure 3. Kinetic model fitting to the synthesis of (A) cetyl laurate, (B) cetyl myristate, (C) cetyl palmitate and (D) cetyl stearate with Lipozyme® RM IM: (●) 0.125, (▼) 0.250, (■) 0.500, (♦) 1.000 g and (-) model. Experimental conditions: 20 g of substrates, 1:1 molar ratio, 70ºC, 350 rpm, 54 L/h N_2 and 21.3 kPa.

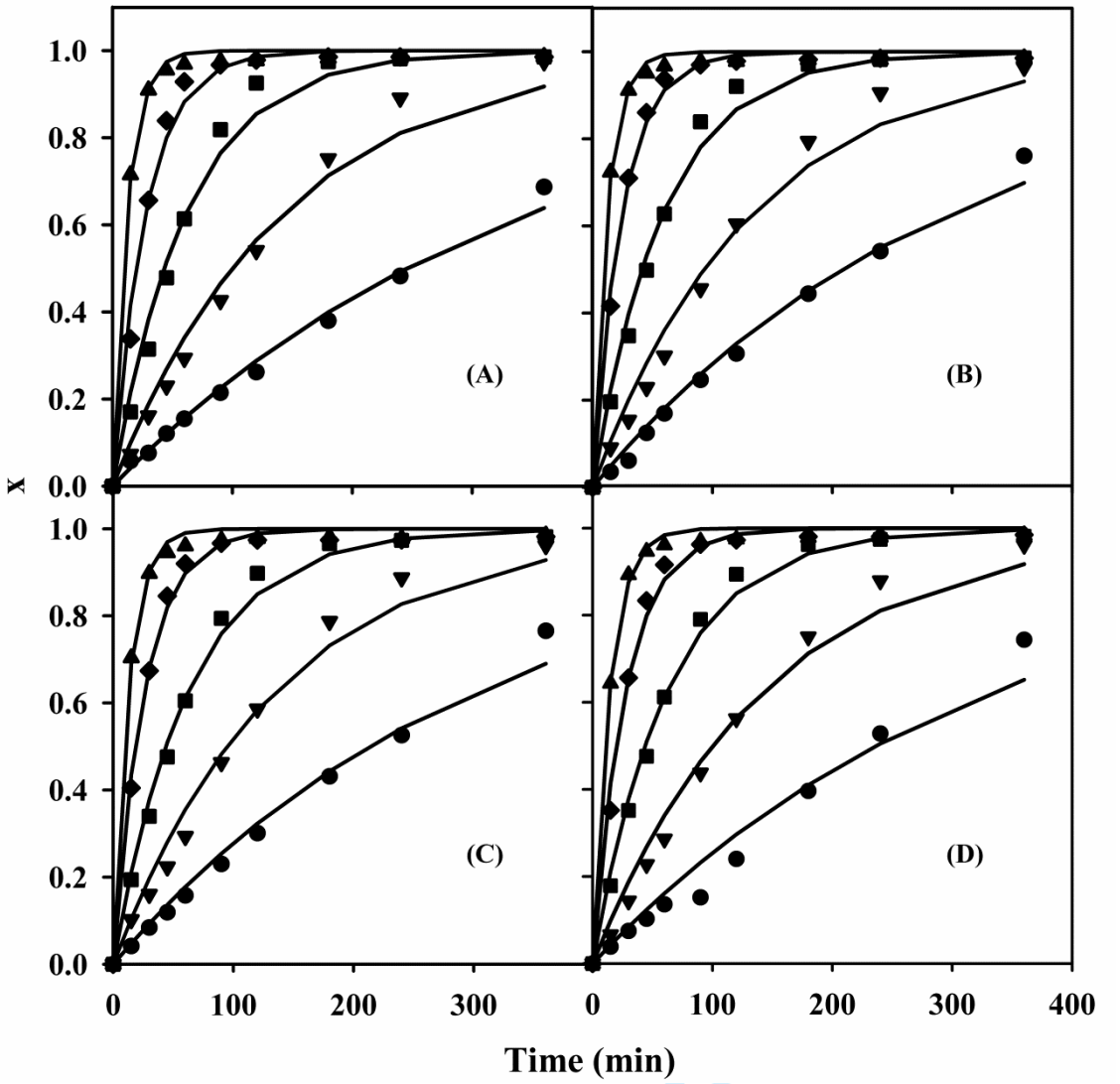

Figure 4. Kinetic model fitting to the synthesis of (A) cetyl laurate, (B) cetyl myristate, (C) cetyl palmitate and (D) cetyl stearate with Lipozyme® TL IM: (●) 0.125, (▼) 0.250, (■) 0.500, (♦) 1.000, (▲) 2.000 g and (-) model. Experimental conditions: 20 g of substrates, 1:1 molar ratio, 70ºC, 350 rpm, 54 L/h N_2 and 21.3 kPa.

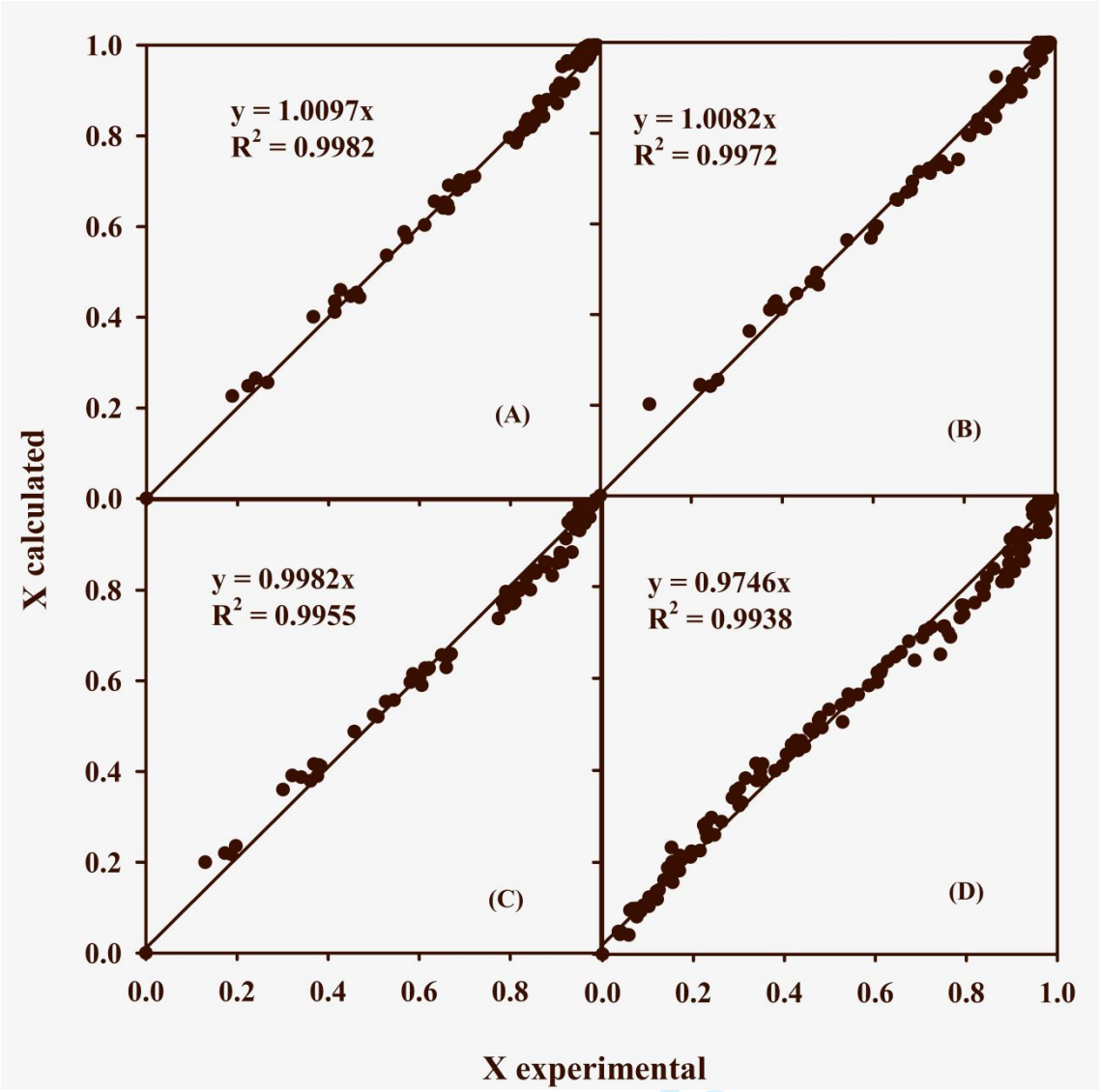

Figure 5. Comparison of experimental conversion values with the ones predicted by the model for the synthesis of cetyl esters with different enzymes: (A) CalB immo Plus (B) Novozym® 435 (C) Lipozyme® RM IM and (D) Lipozyme® TL IM.

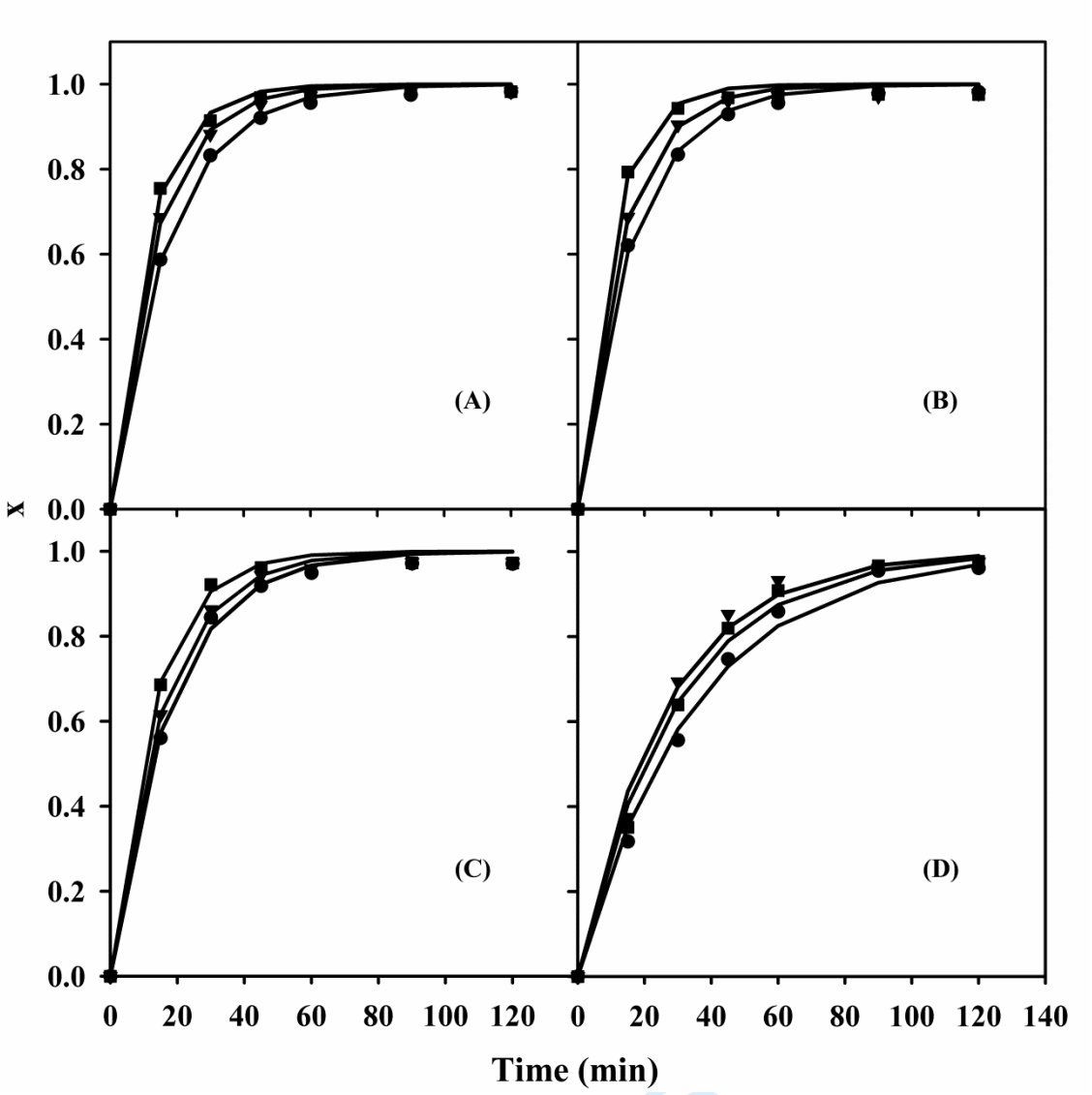

Figure 6. Kinetic model fitting to the synthesis of spermaceti at different temperatures with the optimum amount of biocatalyst (A) 0.5 g CalB immo Plus, (B) 0.5 g Novozym® 435, (C) 0.5 g Lipozyme® RM IM and (D) 1.0 g Lipozyme® TL IM : (●) 60, (▼) 70, (■) 80 ºC and (-) model. Experimental conditions: 20 g of substrates, spermaceti proportions, 350 rpm, 54 L/h N_2 and 21.3 kPa.

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TABLES

Table 1. Kinetic constant values for separately synthesis of cetyl esters.

	Amount (g)	Kinetic constant, k_r (min^{-1})			
		Cetyl laurate	Cetyl myristate	Cetyl palmitate	Cetyl stearate
CalB immo Plus	0.125	0.0190	0.0206	0.0197	0.0171
	0.250	0.0404	0.0391	0.0355	0.0353
	0.500	0.0826	0.0781	0.0696	0.0680
	1.000	0.1309	0.1240	0.1060	0.1116
Novozym® 435	0.125	0.0187	0.0185	0.0197	0.0150
	0.250	0.0426	0.0353	0.0418	0.0352
	0.500	0.0875	0.0829	0.0893	0.0859
	1.000	0.1349	0.1343	0.1340	0.1249
Lipozyme® RM IM	0.125	0.0179	0.0165	0.0163	0.0149
	0.250	0.0329	0.0356	0.0353	0.0318
	0.500	0.0620	0.0605	0.0659	0.0543
	1.000	0.1163	0.1015	0.1074	0.0996
Lipozyme® TL IM	0.125	0.0028	0.0033	0.0033	0.0029
	0.250	0.0069	0.0075	0.0073	0.0069
	0.500	0.0161	0.0169	0.0158	0.0158
	1.000	0.0357	0.0405	0.0380	0.0356
	2.000	0.0814	0.0828	0.0778	0.0694

Table 2. k_r relation with the amount of biocatalyst (m_E) according to equation 23.

		Cetyl laurate	Cetyl myristate	Cetyl palmitate	Cetyl stearate
CalB immo Plus	$1/k_r$ vs $1/m_E$	y= 6.5112x-0.1591 R^2=0.9969	y= 5.8639x+1.7874 R^2=0.9992	y= 5.9681x+3.2892 R^2=0.9982	y= 7.1534x+0.8162 R^2=0.9982
Novozym® 435	$1/k_r$ vs $1/m_E$	y= 6.7061x-1.1983 R^2=0.9923	y= 6.8106x-0.0418 R^2=0.9970	y= 6.3210x-0.3561 R^2=0.9959	y= 8.5979x-3.6126 R^2=0.9906
Lipozyme® RM IM	$1/k_r$ vs $1/m_E$	y= 6.7074x+2.5730 R^2=0.9987	y= 7.2404x+1.5870 R^2=0.9945	y= 7.4863x+0.4365 R^2=0.9956	y= 8.1407x+1.2830 R^2=0.9961
Lipozyme® TL IM	$1/k_r$ vs $1/m_E$	y= 45.774x-21.874 R^2=0.9926	y= 38.755x-14.141 R^2=0.9978	y= 39.544x-13.278 R^2=0.9977	y= 43.974x-18.054 R^2=0.9945

Table 3. Pseudo-first order kinetic constant values and their relation with temperature for the spermaceti synthesis.

	Kinetic constant, k_r (min^{-1})			Arrhenius (Ec. 24)
	303 K	343 K	353 K	
CalB immo Plus	0.0583	0.0744	0.0901	y= -2562.1x+4.858 R^2=0.9974
Novozym® 435	0.0617	0.0768	0.1024	y= -2974.7x+6.1337 R^2=0.9915
Lipozyme® RM IM	0.0567	0.0640	0.0787	y= -1917.3x+2.8724 R^2=0.9716
Lipozyme® TL IM	0.0291	0.0382	0.0346	y= -1047.2x-0.3336 R^2=0.4171

Table 4. Comparison of the weighted average kinetic constants for the synthesis of spermaceti with the corresponding one-step process pseudo-first order constants.

	Compound	Kinetic constant, k_r (min^{-1})	% in espermaceti	Contribution to k_r
CalB immo Plus	Cetyl laurate	0.0826	15.31	0.0127
	Cetyl myristate	0.0781	37.76	0.0295
	Cetyl palmitate	0.0696	38.78	0.0270
	Cetyl stearate	0.0680	8.16	0.0056
	Spermaceti, k_r weighted average (min^{-1})			0.0748
	Spermaceti, k_r model prediction (min^{-1})			0.0744
Novozym® 435	Cetyl laurate	0.0874	15.31	0.0134
	Cetyl myristate	0.0829	37.76	0.0313
	Cetyl palmitate	0.0893	38.78	0.0346
	Cetyl stearate	0.0859	8.16	0.0070
	Spermaceti, k_r weighted average (min^{-1})			0.0863
	Spermaceti, k_r model prediction (min^{-1})			0.0768

Lipozyme® RM IM	Cetyl laurate	0.0620	15.31	0.0095
	Cetyl myristate	0.0605	37.76	0.0229
	Cetyl palmitate	0.0659	38.78	0.0256
	Cetyl stearate	0.0543	8.16	0.0044
	Spermaceti, k_r weighted average (min^{-1})			0.0624
	Spermaceti, k_r model prediction (min^{-1})			0.0640
Lipozyme® TL IM	Cetyl laurate	0.0357	15.31	0.0055
	Cetyl myristate	0.0405	37.76	0.0153
	Cetyl palmitate	0.0380	38.78	0.0147
	Cetyl stearate	0.0356	8.16	0.0029
	Spermaceti, k_r weighted average (min^{-1})			0.0384
	Spermaceti, k_r model prediction (min^{-1})			0.0382

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