KINETIC MODEL FOR FAME PRODUCTION USING IMMOBILIZED LIPASES IN A SOLVENT-FREE SYSTEM

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ABSTRACT
A kinetic model was developed to describe FAME production from refined, bleached and deodorized palm oil (RBDPO) in a solvent-free system, using Thermomyces lanuginosus lipase, in free and immobilized form. The limitations of substrate mass transfer, enzymatic inhibition and discontinuous feeding of alcohol were considered. The kinetic model for the enzyme in free and immobilized form was validated experimentally, under the same process conditions (34 °C, 0.1454 mg protein/g oil, 4.10:1 methanol: oil molar ratio). The kinetic model predicted a FAME content of 73.47 wt % at 9 hours, with a relative error of 0.140% using the enzyme in free form, while the FAME content predicted by the kinetic model was 47.04 wt % at 9 hours with a relative error of 0.026 using the enzyme in immobilized form. The decrease in the percentage of esters using the enzyme in immobilized form was attributed to limitations by external mass transfer.

RESUMEN
Se desarrolló un modelo cinético para describir la producción de FAME a partir de RBDPO (Refined, Bleached and Deodorized Palm Oil) en un sistema libre de solvente, usando la lipasa de Thermomyces lanuginosus, en forma libre e inmovilizada. Se tuvo en cuenta las limitaciones por transf. de masa de los sustratos, inhibición enzimática y alimentación discontinua del alcohol. Se validó experimentalmente el modelo para la enzima en forma libre e inmovilizada, bajo las mismas condiciones de proceso (34 °C, 0.1454 mg proteína/g de aceite, relación molar Metanol: Aceite de 4.1), prediciendo el contenido de FAME en 9 horas, con un error del 0.140% usando la enzima en forma libre, y con un error del 0.026% usando la enzima en forma inmovilizada. La disminución en el porcentaje de ésteres usando la enzima en forma inmovilizada fue atribuida a las limitaciones por transferencia de masa externa.

KEYWORDS / PALABRAS CLAVE
FAME | Immobilized lipase | Kinetic Modeling | RBDPO | Transesterification

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Lipases are versatile enzymes widely used in different types of reactions. However, the use of lipases as their native form is often hampered by several limitations, such as high costs, low operational stability in unusual mediums such as organic media and difficulties in recovery and reuse [9, 10]. These drawbacks could be overcome through the immobilization of lipases [11]. Different procedures have been used to immobilize lipases; however, the physical adsorption on hydrophobic supports has been the most widely used because it is a simple, gentle and cheap protocol to prepare active and robust biocatalysts [10].

Mesoporous silicas are promising candidates for lipase adsorption with respect to the requirements of enzyme carriers, i.e., large surface area, low pore size distribution, well-defined pore geometry, their thermal and mechanical stability and toxicological safety. Furthermore, the surface of silica supports can be chemically modified with various functional groups [9, 10, 11]. Depending on the type of functional group on the surface of the support and the degree of hydrophobicity, different types of interactions between the enzyme and the support can be promoted. Lipases precipitate as a catalytic mechanism known as interfacial activation, where a lipids layer that covers the active site in most lipases is modulated from a closed form (inactive enzyme) to an open form (active enzyme) in the presence of hydrophobic interfaces [10]–[13]. Thus, an adequate immobilization protocol should promote hydrophobic interactions between lipase and support. A successful method for immobilizing lipases on Octyl-silica supports has been reported by different authors [10]–[12], [14], [15]. This method acquires a unique hydrophobic character that can promote the hydrophobic interactions and interfacial activation of lipases. Different authors have independently reported the mass transfer analysis of immobilized enzymes and enzymatic kinetics using the enzyme free form for FAME production [2]–[8], [16]–[20]. Based on this, the described method was used in this study to immobilize a process for the lipase Thermomyces Lanuginosus.

This research integrated the aforementioned studies through a heterogeneous enzymatic kinetic analysis for FAME production. A simultaneous hydrodeoxygenation and alcoholysis mechanism, previously reported in the literature, was used to take into account the products of the intermediate reactions and the limitations by mass transfer when using the enzymes in immobilized form [Sudarmayani 2], [7], [8]. Experimental validation of the proposed model was performed by testing an immobilized lipase from Thermomyces Lanuginosus.

The immobilization was supported functionally with Octyl-groups in accordance with the methodology described by Lima et al. [14], using a mesoporous support [21]. Then, the immobilization process of the enzyme was developed as per the literature [11], [27] through the methodology of preparing a suspension containing the enzymatic solution, previously prepared in a sodium phosphate buffer solution, with a provision of 100 mg protein/g of support, which was stirred for 24 hours at 25°C. Finally, the immobilized support was filtered and washed with deionized water [11].

The characterization of the immobilized support is explained in detail in the work by Castro-Posada [21]. The resulting immobilized support had a load of 56.5 mg of immobilized protein/g and approximated average hydrodynamic activity of 347 U/g of immobilized support [21].

The presence of solvents in the reactive medium entailed the addition of more post-process separation stages, therefore no solvents were used in this study. The factors that were set at fixed values were the amount of water (2% of the oil weight) and the stirring (200 ± 1 rpm).

To avoid the inhibition of the enzyme by high methanol concentration and substrate molar ratio set were at 38 °C and 6, respectively. The higher amount of enzyme used in accordance with the literature on similar enzymatic liquid formulations [22]–[24]. The Michaelis-Menten equation was used to determine the protein content in the enzymatic immobilized [21]. About 50 grams of oil were used in each experimental test. Therefore, the amount of enzyme solution used was 0.556g containing 2.752 mg protein. On the other hand, the protein content of the immobilized support was 56.5mg protein/g immobilized. Thus, in each experimental test applying immobilized enzyme, an amount of 0.128 g of immobilized support was used.

## EXPERIMENTAL DEVELOPMENT

### MATERIALS

| Substance       | Density (g/ml) | Viscosity (cP) |
|-----------------|----------------|---------------|
| RBPO            | 898.08         | 44.68         |
| Methanol        | 788            | 0.46          |
| Water           | 1025.75        | 25.8          |
| Propylene glycol| 1025.75        | 25.8          |

### EXPERIMENTAL DATA

The experimental data that served as input information for the preparation of the kinetic model were obtained through the assembly of independent systems in Erlenmeyers, fed with the same amount of oil, methanol, water and enzyme. The temperature and stirring of every sample was controlled using a thermostatic bath. The reaction was performed at different times (0, 3 and 6 hours) to avoid the inhibition of the enzyme by high methanol concentration in the reactive medium [25], [26]. In each Erlenmeyer, the reaction was stopped by thermal inhibition at 100 °C in accordance with a specified reaction time (3, 4, 6, 8, 10, 12, 18 and 24 hours) [23]. In this study, the same amount of protein was used in free enzyme and immobilized enzyme assays, respectively. The Bradford method was used to determine protein content in enzymatic solutions, while thermogravimetric analysis (TGA) was used to determine protein content in the enzymatic immobilized [21]. About 50 grams of oil were used in each experimental test. Therefore, the amount of enzyme solution used was 0.556g containing 2.752 mg protein. On the other hand, the protein content of the immobilized support was 56.5mg protein/g immobilized. Thus, in each experimental test applying immobilized enzyme, an amount of 0.128 g of immobilized support was used.

### ADJUSTMENT OF KINETIC MODEL PARAMETERS

This was done by using the MATLAB® R2014b program with an optimization subroutine. The method used to adjust the kinetic model parameters consisted of minimizing the sum of the squares of the relative difference between the values of the experimental concentrations and the model for triacylglycerols, diglycerides, monoglycerides and methyl esters, according to the following equation [7], [28]:

\[ \text{Cumulative error} = \sum_{i=0}^{n} \left( C_{\text{experimental}} - C_{\text{estimated}} \right)^2 \]

Where \( C_{\text{estimated}} \) represents the concentration of species i of the model at a time t, while \( C_{\text{experimental}} \) represents the concentration of species i experimentally obtained at the same time t.

### KINETIC MODEL DEVELOPMENT

The kinetic model developed comprised differential and globalized parameters.

### LIMITATIONS BY MASS TRANSFER WITH ENZYME IN FREE FORM

To determine the limiting step of the enzyme-catalyzed process in free form, the mass transfer rate and reaction rate were compared.

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For the estimation of the diffusion coefficients with very diluted liquid mixtures, the Wilke and Chang correlation was used [34]:

\[
D_2 = \frac{2 \times 10^{-6} (0.2 M) 1.5 + 0.1 \lambda}{\mu} \rho_f \gamma_v^n \rho_v^n
\]

(11)

In order to calculate the real diffusivity coefficients, simple expressions can be used as combinations of the coefficients of infinite diffusion for each single species. One of the expressions for binary blends is [34]:

\[
D_{12} = D_{21} = (D_{11} \gamma_v^n D_{22} \gamma_v^n)^{1/2}
\]

(12)

where \( \gamma_v \) and \( \gamma_v \) are the mass fractions of species 1 and 2, respectively.

**KINETICS WITH ENZYME IN FREE FORM**

The overall reaction for FAME production is presented by Jorba, [34]:

\[
TG + 3 Al = 3 ES + G
\]

(13)

Previously, it was indicated that the enzymatic process for FAME production was based on the Ping-Pong Bi Bi mechanism with competitive inhibition of alcohol, using Equation 14 [6],[19],[35]:

\[
v = \frac{v_{\text{max}} |TG| |Al|}{|TG| + |Al| + \frac{k_{mTG} |TG|}{k_{mAl}}} \]

(14)

For the development of the kinetic model with enzyme in free form, a system of equations that represent a hydrolysis/fractionation and alcoholysis process in parallel was used, generated through the Ping-Pong Bi Bi mechanism [7],[8].

The mechanism comprises three basic assumptions:

1. The reaction rate is slow enough for the mass transfer limitations to be negligible.
2. All fatty acids released can be grouped and treated as a single constituent (F).
3. The inhibition reaction of the enzymatic active site by alcohol follows a mechanism of competitive inhibition.

The resulting expressions for the reaction rates involved in hydrolysis/fractionation and alcoholysis process in a parallel mechanism are described by Cherchi [7]:

\[
\frac{dG}{dt} = (V_{nm}[W] + V_{m}[Al][M][E]) [E]
\]

(18)

\[
\frac{dF}{dt} = \left( (V_{nm}[TG] + V_{m}[Al]) [M][W] - V_{m}[Al][F][Al][E] \right)
\]

(19)

\[
\frac{dW}{dt} = \left( (V_{nm}[TG] - V_{m}[Al]) [M][W] - V_{m}[Al][F][Al][E] \right)
\]

(20)

\[
\frac{dE}{dt} = \left( (V_{nm}[TG] + V_{m}[Al]) [W] + V_{m}[Al][F][Al][E] \right)
\]

(21)

\[
\frac{dAl}{dt} = -\frac{dE}{dt}
\]

(22)

This mechanism has 12 unknown parameters, which are \( V_{nm}, V_{m}, V_{mg}, V_{mA}, V_{mAl}, V_{mTG}, K_{mTG}, K_{mA}, K_{mAl}, K_{mE} \), and \( K_{E} \).

This system of equations (Equations 4-12) has advantages over Equation 3 because it makes it possible to differentiate each of the intermediates and by-products that are part of a real process of enzymatic-FAME production, unlike the simplified model, which only considers the overall reaction. In this model, it was assumed that the initial concentration of water in the medium relates to the soluble concentration. For the simulation of the model, discontinuous methanol feed was taken into account at three different times (0, 3, and 6 hours) until a kL/Jo methyl ester oil rate was achieved.

**EXTERNAL MASS TRANSFER**

After preparing the mathematical model for the process and identification of kinetic parameters without further effects of mass transfer limitations, the next step was to identify the mass transfer limitations for an immobilized enzyme system.

Figure 1 shows the adsorption isotherm of the immobilization support. The elongated form of the hysteresis cycle could correspond to a mesoporous material with very uniform cylindrical pores, but could also relate to mesoporosity generated in the interparticle space [36]. The average pore size of the mesoporous, which was calculated using the DFT method, was 4 nm. On the other hand, the Thermomyces lanuginosus lipase has a volume of 5 nm x 6.5 nm x 5.5 nm [37]. Thus, it was deemed that the immobilization of the enzyme occurred only off the outside of the pores and in the interparticle space of the material [36]. Therefore, in this case, internal limitations by mass transfer were considered negligible.

Under these conditions, the rate of consumption of triglycerides, methanol and water in the oleic phase can be expressed by the following equations [2]:

\[
\frac{dW}{dt} = -\sigma_{K_W} a_1 (TG) - |W| \]

(24)

\[
\frac{dA}{dt} = -\sigma_{K_A} a_1 ([Al] - |Al|) \]

(25)

\[
\frac{dF}{dt} = -\sigma_{K_W} a_1 ([Al] - |Al|) \]

(26)
Parameter \( \sigma \) makes it possible to modify the mass transfer coefficients as the reaction time increases, representing the growth of the glycerol layer around the immobilization support. The value of parameter \( \sigma \) takes the value of 1 at time zero and the value of zero at infinite time, in accordance with the following equation (21):

\[
\sigma = e^{-\alpha t}
\]  

(27)

The mass transfer coefficients \( \alpha \), were calculated in a manner similar to those for enzymes in free form, assuming that the immobilization support has a spherical shape. The system for calculating the mass transfer coefficients of each species was:

\[
Ar = Re = Sc = Sh = \alpha_i
\]

The experimental tests to ascertain particle size distribution of the support were not carried out in this study. It was deemed that the diameter of the immobilization support was 200 nm and spherical in shape, according to the supplementary information presented in the paper by Logothetan et al., used in the synthesis of the immobilization support [38]. Experimentally, it was found that the value for density of the porous support was 572 kg/m³ [21]. Further, to calculate the Reynolds number, the immobilization support was assumed to be a free-moving sphere inside a stirred vessel. The Archimedes number \( Ar \) was calculated for this situation and then the Reynolds number \( Re \) was calculated according to the value taken from the Archimedes number [32],[33]. The Schmidt number \( Sc \) was then calculated, considering the diffusivity of triglycerides, methanol, and water in the glycerol.

Once the equations describing the mass transfer rate from the oleic phase to the solid-liquid interface were obtained, the next step was to couple these expressions to the reaction rate system that was previously illustrated for the enzyme in free form. The reactions were developed over the solid-liquid interface and the concentrations \( T \), \( Al \), and \( W \), change according to the following Equations (2):

\[
\frac{d[T]}{dt} = \left( k_R Al[T] - [T] \right) - \left( \frac{V_A[T] + V_A[Al][T][E]}{[E]} \right)
\]

(28)

\[
\frac{d[Al]}{dt} = \left( \frac{k_R Al[Al] - [Al]}{[E]} \right) - \left( \frac{V_A[Al][T]}{[E]} + V_A[Al][E][Al][E] \right)
\]

(29)

\[
\frac{d[W]}{dt} = \left( k_R Al[W] - [W] \right) + \left( \frac{V_A[W][E][E][Al][E]}{[E]} \right) - \left( \frac{V_V[W][W]}{[E]} + V_V[Al][E][Al][E] \right)
\]

(30)

In Equations 17, 18, and 19, the first terms enclosed in brackets correspond to mass transfer rates, while the second terms enclosed in brackets correspond to reaction rates. The other chemical reactions that are part of the reaction mechanism were modified only in terms of the concentrations at which the reactions were carried out, i.e., the concentrations at the interface. Thus, \( [T] \) was changed by \( [T], [Al] \) by \( [Al] \) and \( [W] \) by \( [W] \), corresponding to the concentrations at the interface for the triglycerides, alcohol, and water respectively.

\[\text{Parameter } \sigma \text{ makes it possible to modify the mass transfer coefficients as the reaction time increases, representing the growth of the glycerol layer around the immobilization support.} \]

\[\text{The equations describing the mass transfer rate from the oleic phase to the solid-liquid interface were developed.} \]

\[\text{The experimental tests to ascertain particle size distribution of the support were not carried out in this study.} \]

\[\text{The Archimedes number } Ar \text{ was calculated for this situation and then the Reynolds number } Re \text{ was calculated according to the value taken from the Archimedes number [32],[33].} \]

\[\text{Once the equations describing the mass transfer rate from the oleic phase to the solid-liquid interface were obtained, coupled with the reaction rate system, the next step was to calculate the mass transfer coefficients of each species.} \]

\[\text{The chemical reactions that are part of the reaction mechanism were modified only in terms of the concentrations at the interface.} \]

\[\text{The parameters were calculated using the equations provided.} \]

\[\text{The model was validated by calculating the relative error rate for the prediction of FAME content during 9 hours, at a temperature of 34 °C, 4.10:1 methanol: oil molar ratio, 0.1454 mg protein/g oil, 2% weight of water relative to the oil, and 200rpm working conditions.} \]

\[\text{These experimental conditions were produced in triplicate.} \]

\[\text{The relative error rate for FAME (E) was calculated by González [29] as:} \]

\[E(\%) = \frac{\text{FAME}_{\text{Experimental}} - \text{FAME}_{\text{Model}}}{\text{FAME}_{\text{Experimental}}} \times 100 \]  

(34)

\[\text{The model generated the FAME concentration of 73.47 %, while experimentally it was found that the FAME concentration under these same process conditions resulted in a value of 73.36±0.33%.} \]

\[\text{The relative error rate for FAME was 0.14 %}. \]

\[\text{The percentage of FAME obtained when using the enzyme in free form was compared to the enzyme in immobilized form for the FAME production process under the same processing conditions (34 °C, 4.10:1 mg protein/g oil, 4.10:1 methanol: oil molar ratio, and 9 hours reaction), as shown in Table 4.} \]

\[\text{It was found that the percentage of FAME obtained decreased from 73.36±0.33 % when using the enzyme in free form, to 74.03±4.63 % when using the immobilized enzyme.} \]

\[\text{After performing a comparison of means with a significance level of 0.05, the decrease in FAME percentage was found to be statistically significant at the 95 % level and was attributed to the external mass transfer limitations imposed by the immobilization support [38].} \]
The mass transfer coefficients calculated for triglycerides, methanol and water from the oleic phase to the solid-liquid interface were:

\[ k_F = 8.51 \times 10^{-5} \text{ m/s} \]  
\[ k_M = 5.26 \times 10^{-8} \text{ m/s} \]  
\[ k_W = 6.61 \times 10^{-8} \text{ m/s} \]

The value of parameter \( a \) in Equation 27 was adjusted in accordance with experimental data for the immobilized enzyme system at a value of 2.61 \( 1^\text{h} \). The expected dynamic behavior for the concentrations of FAME, mono-esters, diglycerides, triglycerides and free fatty acids in the oleic phase is shown below.

Figure 4 illustrates a fast rate of triglyceride consumption in the first instants of the simulation (0.2 hours), while the increase in the concentration of the other chemical species is slow. This was attributed to the mass transfer phenomenon in the early stages of the process. Subsequently, it was observed that triglyceride consumption increased exponentially up to the point at which, on the one hand, it increased in the concentration of the other species, most likely due to the formation of the layer of glycerol and the progress of the reactions.

The validation of the model with enzyme in immobilized form as shown in Figure 4 demonstrated that the mass transfer limitations of the aqueous phase to the oleic phase using the enzyme in free form were negligible, as are the limitations by mass transfer to the support using the enzyme in immobilized form.

After analyzing the mass transfer limitations involved in the FAME production enzymatic process separately, it can be concluded that the mass transfer limitations of the aqueous phase to the oleic phase using the enzyme in free form were negligible, as are the limitations by mass transfer to the support using the enzyme in immobilized form.

Moreover, it was possible to fit an alkenic model to an enzyme in free form process, with a cumulative error of 4.4%. This indicates that the cumulative error obtained for the free enzyme model had a better fit than the original model based on literature, which had a value of 34.39. The model was validated by the FAME content obtained experimentally using the enzyme in free form. The prediction error for the 9th prediction of the kinetic model with enzyme in free form was 0.14 %.

Finally, a model was developed for the prediction of kinetics with the enzyme in immobilized form, in which mass transfer limitations were completely attributed to the external part of the immobilization support, due to the formation of a layer of glycerol around itself. The model was validated by predicting the FAME content obtained experimentally using the enzyme in immobilized form during the 9 hours. The prediction error for the kinetic model with immobilized enzyme was 0.03%.

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| Symbol | Description |
|--------|-------------|
| [FAME] | Fatty acid methyl esters |
| [CCD] | Central composite design |
| [ε]   | Relative error rate (%) |
| [RBDPO] | Refined, Bleached and Deodorized Palm Oil |
| [RSM] | Response surface methodology |
| [N_A] | Mol transfer rate of component A (mol s⁻¹) |
| [C_A] | Concentration of component A in the aqueous phase (mol m⁻³) |
| [C_mD] | Concentration of component A in the oleic phase (mol m⁻³) |
| [α]   | Distribution or partition coefficient |
| [K_mA] | Global coefficient for mass transfer based on the concentrations of the species in the aqueous and oleic phase (m s⁻¹) |
| [g]   | Gravitational constant (9.8 m s⁻²) |
| [ρ_m] | Density of the oleic phase (kg m⁻³) |
| [µ_m] | Viscosity of the oleic phase (kg m⁻¹ s⁻¹) |
| [d]   | Diameter of the sphere formed by the aqueous phase (m) |
| [µ_a] | Viscosity of the aqueous phase (kg m⁻¹ s⁻¹) |
| [ρ_a] | Density of the aqueous phase (kg m⁻³) |
| [D_m] | Water diffusivity in the oleic phase (m² s⁻¹) |
| [D_a] | Diffusion coefficient of species 1 (solute) present at an infinitely low concentration in species 2 (solvent) (m² s⁻¹) |
| [M]   | Molecular weight of the solvent (g mol⁻³) |
| [T]   | Temperature (K) |
| [µ_s] | Solvent viscosity (kg m⁻¹ s⁻¹) |
| [V_s] | Solute molar volume at its normal boiling point (cm³ mol⁻¹) |
| [ϕ]   | Solvent-associated factor (2.26 for water, 1.9 for methanol, 1.5 for ethanol and 1 for non-associated solvents such as hydrocarbons) |
| [Sh]  | Sherwood Number |
| [Re]  | Reynolds number |
| [Sc]  | Schmidt number |
| [Ar]  | Archimedes number |
| [TG]  | Triglycerides (mol m⁻³) |
| [A]   | Alcohol (mol m⁻³) |
| [E]   | Esters (mol m⁻³) |
| [G]   | Glycerol (mol m⁻³) |
| [D]   | Diglycerides (mol m⁻³) |
| [M]   | Monoglycerides (mol m⁻³) |
| [F]   | Free fatty acids (mol m⁻³) |
| [W]   | Water (mol m⁻³) |
| [E]   | Free enzyme concentration (g_enzyme g_Oil phase⁻¹) |
| [E_c] | Total enzyme concentration (g_enzyme g_Oil phase⁻¹) |
| [v]   | Reaction rate (mol m⁻³ min⁻¹) |
| [V_max] | Maximum reaction rate (mol m⁻³ min⁻¹) |
| [k_mA] | Apparent Michaelis constant for triglycerides (mol m⁻³) |
| [K_mA] | Apparent Michaelis constant for alcohol (mol m⁻³) |
| [k]   | Inhibition constant for alcohol (mol m⁻³) |
| [V_tG] | Triglyceride hydrolysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_mG] | Diglyceride hydrolysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_mM] | Monoglyceride hydrolysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_tD] | Triglyceride alcoholysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_mD] | Diglyceride alcoholysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_mM] | Monoglyceride alcoholysis rate constant (m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [V_tE] | Constant rate of esterification(m³ g_Oil phase mol⁻¹ h⁻¹ g_enzyme⁻¹) |
| [K_mT] | Equilibrium constant for the enzyme-triglyceride complex (m³ mol⁻¹) |
| [K_mD] | Equilibrium constant for the enzyme-diglyceride complex(m³ mol⁻¹) |
| [K_mM] | Equilibrium constant for the enzyme-monoglyceride complex (m³ mol⁻¹) |
| [K_mF] | Equilibrium constant for enzyme-free fatty acid complex (m³ mol⁻¹) |
| [K]   | Inhibition constant mol m⁻³ |
| [a]   | Support specific interfacial area (m²) |
| [k_s] | Triglycerides mass transfer coefficient (m s⁻¹) |
| [k_m] | Alcohol mass transfer coefficient (m s⁻¹) |
| [k_w] | Water mass transfer coefficient (m s⁻¹) |
| [σ]   | Non-dimensional parameter for the decrease of mass transfer coefficients (0-1) |
| [α]   | Parameter (h⁻¹) |