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Kinetic characterisation of enzymatic esterification in a solvent system: adsorptive control of water with molecular sieves

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Abstract

The kinetics of enzymatic esterification of glycerol with oleic acid, in equimolar ratio, catalyzed by immobilized *Mucor miehei* lipase in a solvent system in the presence of the molecular sieves was carried out at 37°C at different *Lipozym* and solvent (*n*-hexane) concentrations and the molecular sieve contents were studied in a batch stirred-tank reactor (BSTR). The reactions were followed by the determination of reaction conversions during 45 h. The experimental data of enzymatic esterification of glycerol with oleic acid in a solvent system in the presence of molecular sieves showed minimal deviation from the calculated value in the irreversible second order kinetic model. On the basis of the experimental data, we found an empirical correlation between concentrations of *Lipozym*, concentrations of solvent (*n*-hexane), contents of the molecular sieve and the reaction rate constant, k_1.

Keywords: Lipase-catalyzed esterification; Long-chain fatty acid esters; Molecular sieves; *n*-Hexane; Reaction kinetics

1. Introduction

The use of mono- and diacylglycerides as non-ionic emulsifiers in the food and pharmaceutical industries as well as their utilisation as synthetic intermediates in the chemical industry have been growing in recent years [1,2].

Lipase-catalyzed esterification in organic solvents is a reaction where water plays a crucial role. A minimal amount of water is necessary for the enzyme to ensure its optimal conformation and then optimal activity [3,4]. An excess of water decreases the enzyme activity both from kinetic and thermodynamic points of view [5]. The optimal level of enzyme hydration to reach optimal activity appears to be enzyme-dependent [6,7]. Generally, enzyme-catalyzed synthesis is commonly carried out in water, where water in solvents drives the reaction equilibrium in the direction of hydrolysis [8–10]. Non-aqueous organic solvents system has an advantage over aqueous systems in synthesizing glycerides because water can be eliminated.

The conversion rate of esterification can be favoured by the adsorption of water produced during

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the esterification [11,12]. Adsorbents such as alumina, silica gel and zeolites are effective in removing water from organic solvents. The fact partly explaining their superior drying ability is that molecular sieves cannot co-adsorb large hydrocarbon molecules as do common silica and alumina adsorbents. The performance of these adsorbents depends to some extent on the procedure. Some authors will attempt to compare adsorbents by means of surface area measurements dependent on adsorbents particle size [13–15]. When the 3-Å molecular sieve was added as a desiccant, the enzyme was inactivated presumly due to stripping of essential water [16].

In this work, we used the optimal particle size of the 5-Å molecular sieve because this particle size did not decrease enzyme activity.

In this way, we developed protocols for the synthesis of acylglycerols containing oleic acid. The protocol involves the use of 1,3-specific immobilized lipase from *Mucor miehei* in a solvent system (*n*-hexane) at 37°C with the 5-Å molecular sieve to extract initial water in the solvent and water formed during the reaction.

The reaction kinetics model was determined on the grounds of lipase-catalyzed reaction in batch-stirrer tank reactor.

Our attention was focused on the mechanisms involved in the utilisation of the 5-Å molecular sieve. Various experiments at different concentrations of *Lipozym*, molecular sieve contents and solvent concentrations were examined in equimolar ratio of glycerol and oleic acid. The method is recomended for synthesizing preferentially monoglycerides at high conversion.

2. Experimental

2.1. Materials

Immobilized lipase from *M. miehei*, *Lipozym*, from Fluka (activity 62 U/g; Buchs, Switzerland) was selected to catalyze the reactions. Glycerol was purchased as a technical product of 95% w/w purity from Kemika (Zagreb, Croatia); oleic acid and *n*-hexane were selected from Fluka (Buchs, Switzerland); 5-Å molecular sieve (activated) was selected from Supelco (PA, Bellafonte, USA).

2.2. Equipment and procedure

LR-A 250 IKA laboratory reactor with DTM 11 IKATRON temperature control was filled with an equimolar concentration of reactants. Total reaction mixtures was 75 ml. Reactions were carried out at 37°C for 45 h under atmospheric pressure in the presence of 1000, 3000, 4000 mg of the molecular sieve; 1.60, 6.67, 13.33 mg/ml of *Lipozym* and 0.2, 0.5, 0.8 v/v of *n*-hexane. The reactions were followed-up by taking a double sample at examined reaction times. The conversion of oleic acid was determined by using the pH-stat titrating method.

3. Results and discussion

This study examined the effect of removal of water formed during an enzymatic esterification in a batch stirred-tank reactor (BSTR). In order to show the effect of molecular sieves on this kind of reaction, a model lipase-catalyzed reaction esterification of glycerol with oleic acid in a solvent system with 1,3-specific lipase from *M. miehei*, was chosen. We supposed a pseudohomogenic system without diffusion limitation.

Preliminary reactions were carried out with and without the use of the molecular sieve in order to compare the results. The esterification of glycerol with oleic acid in the presence of the molecular sieve was performed with 6.67 mg/ml of *Lipozym*, 0.5 ml/ml *n*-hexane and 3000 mg of the molecular sieve at 37°C. Conversions of oleic acid (X_A) increased more in the solvent system with the molecular sieve than in the solvent system without the molecular sieve (Fig. 1). We observed that the decrease in conversion (X_A) and water content in the solvent resulted in a decrease of enzymatic activity.

3.1. Reaction kinetics

The products of 1,3-specific lipase-catalyzed esterification of glycerol with oleic acid are mixtures

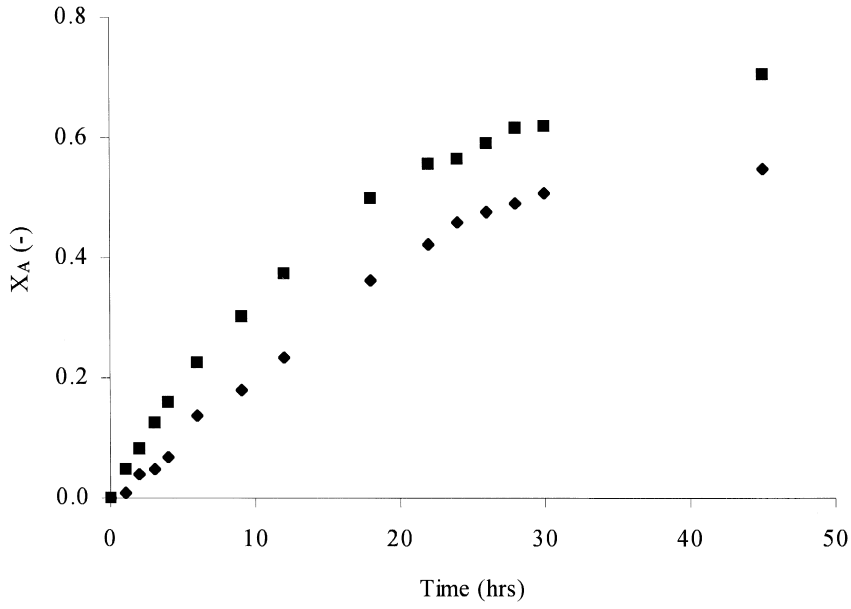

Fig. 1. Conversion of oleic acid (X_A) in esterification of glycerol with oleic acid in the solvent system with 0.5-ml/ml *n*-hexane, 6.67 mg/ml of *Lipozym* at 37°C; (■) — 3000 mg of molecular sieve; (♦) — without molecular sieve.

of 1(3)-monoolein and 1,3-diolein. Considering the esterification as an equilibrium reaction, the following simple reaction scheme can be suggested for the lipase-catalyzed reaction.

$$A + B \overset{k_1/k_2}{\leftrightarrow} R + S \quad (1)$$

where A is the free fatty acid and B is the glycerol, R and S are monoolein and water, respectively. $k_1$is the overall rate constant of the esterification and k_2 is the overall rate constant of the hydrolysis.

However, for esterification reactions, it is desirable to shift the position of thermodynamic equilibrium by removal of the water produced by the reaction in the presence of molecular sieves.

When $C_{S0} = 0$, Eq. (1) can be given as

$$-\frac{dC_A}{dt} = r_A = k_1 C_A C_B \quad (2)$$

where C_{S0} is initial concentration of water, C_A is concentration of oleic acid and C_B is concentration of glycerol at any time (t).

When $C_A = C_B$, Eq. (2) can be given as

$$-\frac{dC_A}{dt} = r_A = k_1 C_A^2 \quad (3)$$

This reaction scheme leads to the following kinetic model of the second order irreversible reaction [17–19].

The conversion (X_A) of the oleic acid at any time (t) is:

$$X_A = \frac{C_{A0} - C_A}{C_{A0}} \quad (4)$$

where C_{A0} is initial concentration of oleic acid.

Combining Eqs. (3) and (4) can be expressed in term of conversion as:

$$-\frac{dX_A}{dt} = r_A = k_1 C_{A0}(1 - X_A)^2 \quad (5)$$

Considering the fact that the content of the 5-Å molecular sieve (C_{MS}), the concentration of *Lipozym* (C_E) and the concentration of *n*-hexane (V_H) influence the k_1 constant, the following empirical relationships can be proposed;

$$k_1 = 0.1096 * \ln\left(-\frac{2.6717}{C_E}\right) \quad (6)$$

$$k_1 = 0.101336 - 0.06235 * V_H \quad (7)$$

$$k_1 = 0.8352 * \ln\left(-\frac{0.5101}{C_{MS}}\right) \quad (8)$$

Calculated values of empirical relationships are in good agreement with the experimental data for the investigated molecular sieve contents, concentrations of *Lipozym* and *n*-hexane concentrations range.

Conversions of oleic acid (X_A) on the basis of experimental data and the kinetic model (Eq. (5)), in the solvent system with 6.67 mg/ml of *Lipozym*, 0.5-ml/ml *n*-hexane at 37°C and at different contents of the molecular sieve are shown in Fig. 2. This figure also shows that the conversion of oleic acid (X_A) depends on the thermodynamic activity of water at different adsorbent contents. But after the examined limit content of the molecular sieve was reached (4000 mg), no better results were obtained. Therefore, we concluded that the tested content of the molecular sieve adsorbed the total amount of free water in the system. The content of the molecular sieve, which was used in the reaction and effectively adsorbed the water produced during the reaction, was 3000 mg.

A simple mechanism of the action of silica gel [20] can be proposed in this system with molecular sieve, too. In this mechanism, the glycerol accumulated on the enzyme creates a polar barrier between the bulk media and the enzyme, limiting the substrate and product diffusion. The presence of *n*-hexane could force this polar barrier to form. The silica gel (in our work the molecular sieve) in the reaction behaves as a "reservoir" for the glycerol, preventing the enzyme from being blocked and allowing the reaction to take place. These results suggest that the transport of glycerol is possible by direct contact between the "reservoir" of glycerol and immobilized lipase.

Conversions of oleic acid (X_A) on the basis of experimental data and the kinetic model, in the solvent system with 3000 mg of the molecular sieve, 0.5-ml/ml *n*-hexane at 37°C and at different concentrations of *Lipozym* are presented in Fig. 3. The increase in the concentration of *Lipozym* increased the conversion of oleic acid (X_A). The higher concentration of *Lipozym* resulted in a higher conversion of oleic acid (X_A) and produced water, too.

One way to decrease the polarity of the reaction medium is to add a nonpolar solvent, such as *n*-hexane in the system. Conversions of oleic acid (X_A) on the basis of experimental data and the kinetic model, in the solvent system with 6.67 mg/ml of *Lipozym*, 3000 mg of the molecular sieve at 37°C and at different concentrations of *n*-hexane, are pre-

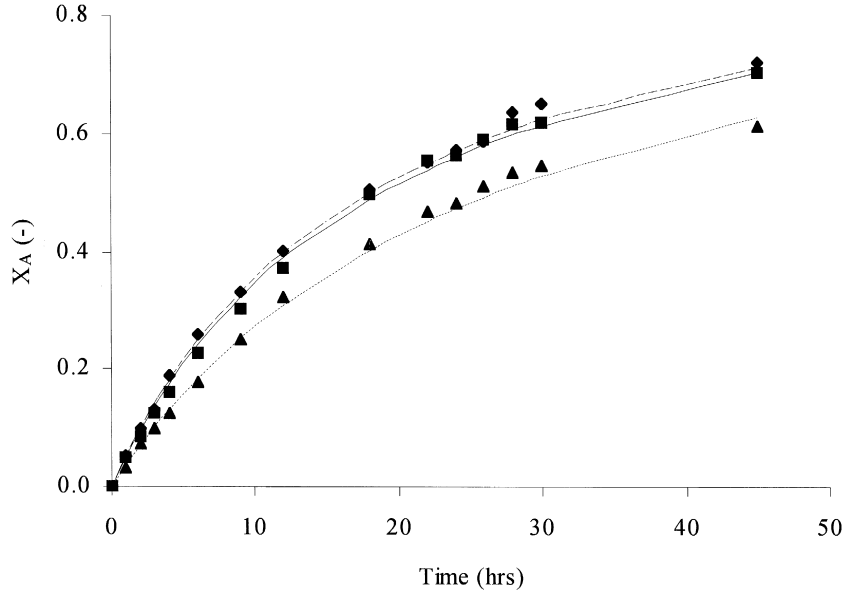

Fig. 2. Conversion of oleic acid (X_A) on the basis of experimental data and the kinetic model (Eq. (5)), in the solvent system with 6.67 mg/ml of *Lipozym*, 0.5-ml/ml *n*-hexane at 37°C and at different contents of the molecular sieve added into 75 ml of reaction mixture. Experimental data are: (▲) — 1000 mg; (■) — 3000 mg; (♦) — 4000 mg. Lines are calculated values.

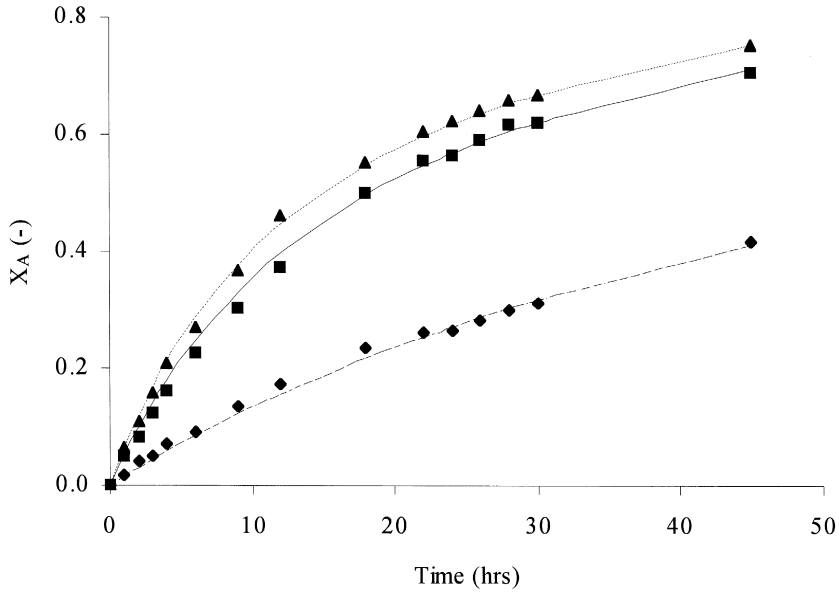

Fig. 3. Conversion of oleic acid (X_A) on the basis of experimental data and the kinetic model (Eq. (5)), in the solvent system with 3000 mg of the molecular sieve, 0.5-ml/ml n-hexane at 37°C and at different concentrations of *Lipozym*. Experimental data are: (♦) — 1.60 mg/ml; (■) — 6.67 mg/ml; (▲) — 13.33 mg/ml. Lines are calculated values.

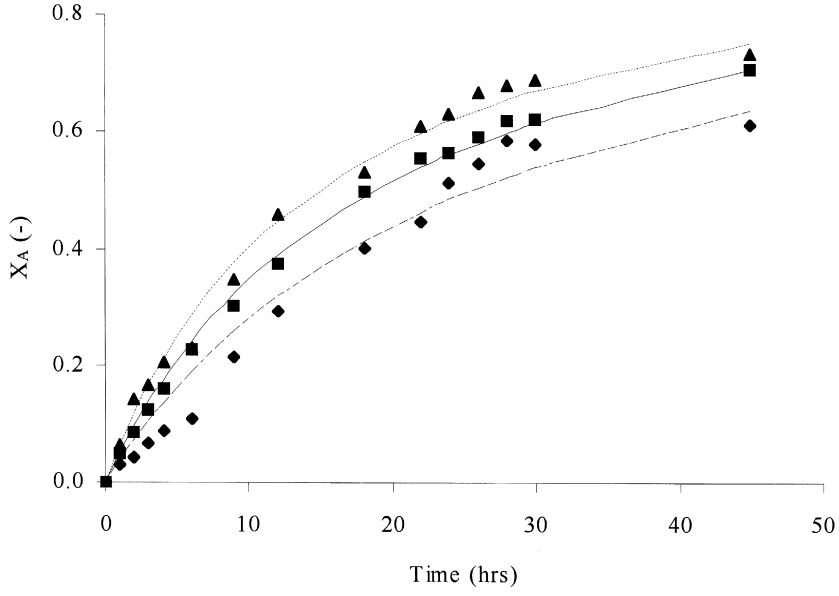

Fig. 4. Conversion of oleic acid (X_A) on the basis of experimental data and the kinetic model (Eq. (5)), in the solvent system with 6.67 mg/ml of *Lipozym*, 3000 mg of the molecular sieve at 37°C and at different concentrations of *n*-hexane. Experimental data are: (▲) — 0.2 ml/ml; (■) — 0.5 ml/ml; (♦) — 0.8 ml/ml. Lines are calculated values.

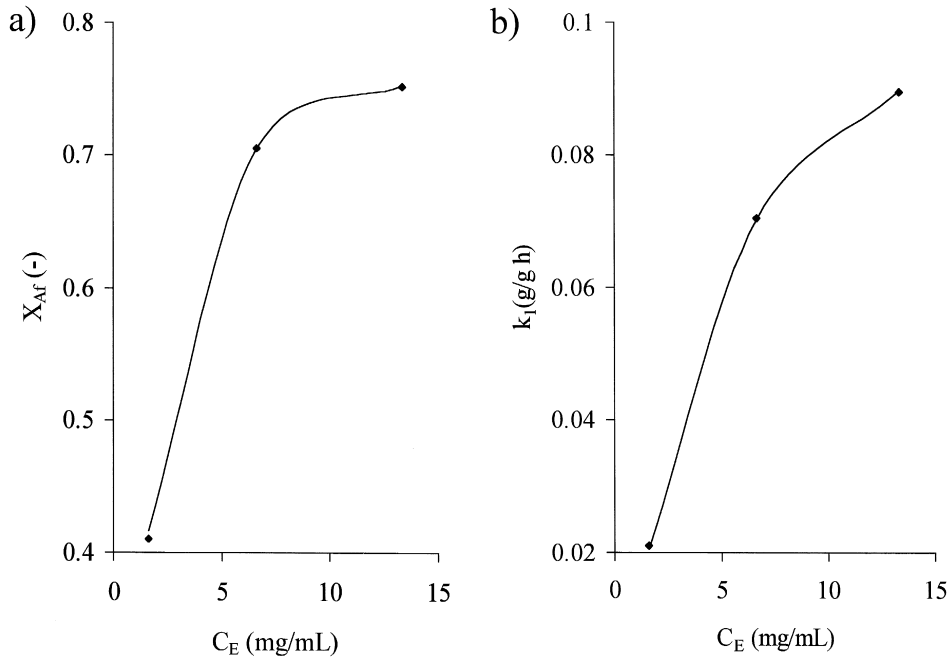

Fig. 5. Dependence of experimental data and simulation data on (a) conversion of oleic acid monitored for 45 h (X_{Af}), and (b) kinetic constant (k_1) at different concentrations of *Lipozym*, in the solvent system, with 0.5 ml/ml *n*-hexane, 3000 mg of the molecular sieve, at 37°C; markers (♦) are experimental data and line is calculated value.

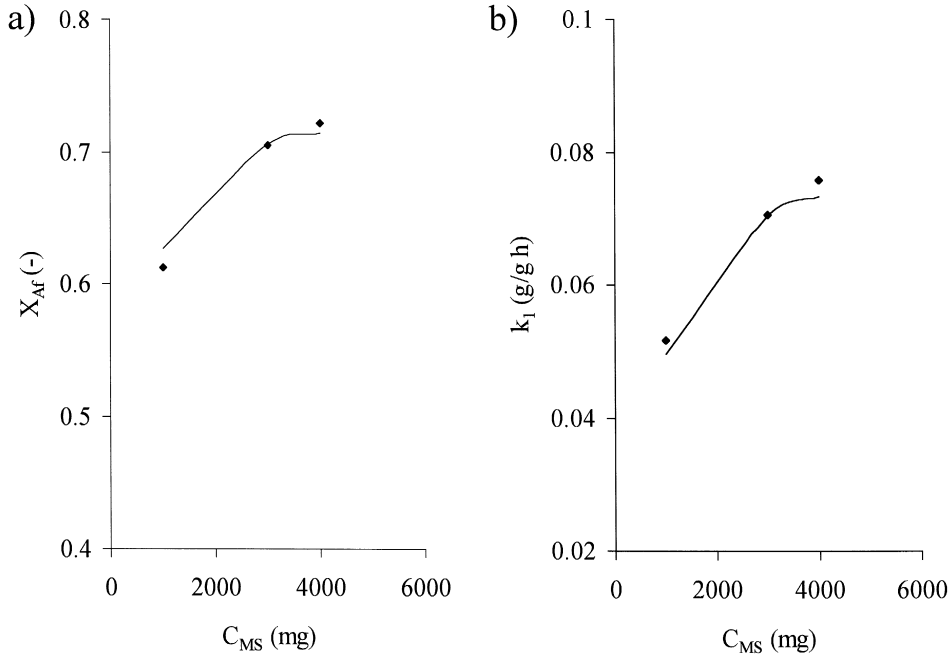

Fig. 6. Dependence of experimental data and simulation data on (a) conversion of oleic acid monitored for 45 h (X_{Af}), and (b) kinetic constant (k_1) at different concentrations of the molecular sieve, in the solvent system, at 0.5 ml/ml of *n*-hexane with 6.67 mg/ml of *Lipozym*, at 37°C; markers (♦) are experimental data and line is calculated value.

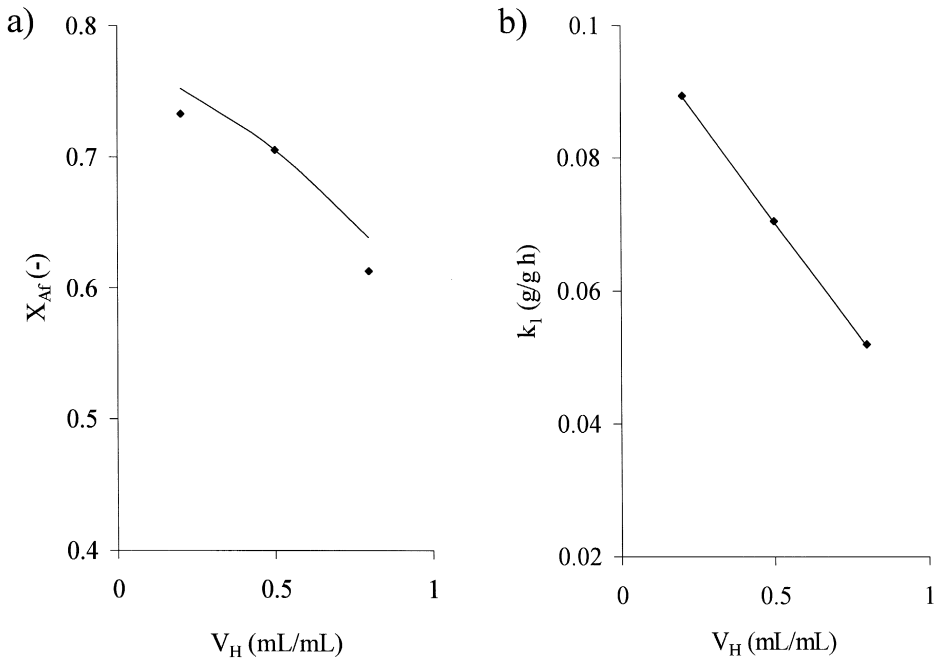

Fig. 7. Dependence of experimental data and simulation data on (a) conversion of oleic acid monitored for 45 h (X_{Af}), and (b) kinetic constant (k_1) at different concentrations of *n*-hexane, in the solvent system with 6.67 mg/ml of *Lipozym*, 3000 mg of the molecular sieve at 37°C; markers (♦) are experimental data and line is calculated value.

sented in Fig. 4. In our experiments, the decrease in *n*-hexane concentration increased the oleic acid (X_A) conversion.

The tested kinetic model (Eq. (5)) of second order irreversible reaction provides an adequate description of the process and experimental data are in a good agreement with the kinetic model (Figs. 2–4).

In test limits, the increase in the concentrations of *Lipozym*, which increased the conversion of oleic acid monitored after 45 h, (X_{Af}) and the esterification constant k_1 are shown in Fig. 5a and b. The increase in the contents of the molecular sieve increased the conversion of oleic acid monitored after 45 h (X_{Af}) and the esterification constant k_1, which are presented in Fig. 6a and b. The increase in *n*-hexane concentration decreased the conversion of oleic acid monitored after 45 h (X_{Af}) and the esterification constant k_1 as shown in Fig. 7a and b.

The observed and calculated constants values of the constant k_1 are in a good agreement as shown in Figs. 5b–7b.

Table 1 shows the estimated values of the rate constant k_1 and the conversions of oleic acid monitored after 45 h (X_{Af}) calculated on the basis of determined model (Eq. (5)) and empirical correlations (Eqs. (6)–(8)).

Table 1
Estimated value of the rate constant (k_1) and conversions of oleic acid monitored for 45 h (X_{Af}) calculated on the basis of the determined model (Eq. (5)) and empirical correlations (Eqs. (6)–(8))

	C_E (mg/ml)	V_H (ml/ml)	C_{MS} (mg)	k_1 (g/g h)	X_{Af} (g/g)
1	1.60	0.5	3000	0.0397	0.4160
2	6.67	0.5	3000	0.0705	0.7116
3	13.3	0.5	3000	0.0897	0.7516
4	6.67	0.5	1000	0.0495	0.6125
5	6.67	0.5	4000	0.0734	0.7215
6	6.67	0.2	3000	0.0889	0.7522
7	6.67	0.8	3000	0.0514	0.6130

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