KINETICS OF PALM OIL HYDROLYSIS BY LIPASE IMMOBILIZED IN A HOLLOW FIBER MEMBRANE REACTOR

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Attractive features of lipase systems include versatility, substrate selectivity, regioselectivity, enantioselectivity and catalysis at ambient temperatures and pressures (Jaeger et al., 2002). To fully exploit the technical and economical advantages of lipases, it is recommended to use them in an immobilized form to reduce the cost and poor stability of the free lipase. Biphasic membrane reactors with lipase immobilized in the membrane present a quite interesting alternative for these enzymatic processes since the enzyme–membrane system acts simultaneously as an interfacial catalyst, a phase contactor and a phase separator (Giorno et al., 2000). Therefore, the use of biphasic membrane reactors with immobilized lipase is highly attractive, especially when hollow fiber reactor configuration is used, since it provides the highest surface to volume ratio without the need for membrane support (Balcao et al., 1998). Several authors have studied lipase immobilization and fat and oil hydrolyses in hollow fiber reactors (Snape et al., 1996). In most of these studies, reactor operating regimes and reaction kinetics were not thoroughly investigated and the *Michaelis–Menten* approach was commonly used. However, the reaction kinetics is more complex than the commonly assumed one–substrate one–product irreversible reaction. In addition, diffusional limitations should be eliminated and the intrinsic kinetics of enzymatic catalysis in this system should be measured.

In a recent paper, we have investigated palm oil hydrolysis by lipase from *Candida rugosa* in a biphasic oil/aqueous hollow–fiber membrane reactor (Knezevic et al., 2004). We have studied the reactor operating regime with respect to flow patterns in reactor zones, lipase desorption and hydrolysis rates and determined the optimal reactor operating regime for palm oil hydrolysis. In the present work, the kinetics of palm oil hydrolysis by immobilized lipase in the membrane reactor under optimal flow conditions was investigated and compared to the results obtained for free lipase in a microemulsion reaction system. In addition, stability of the immobilized enzyme was tested in several hydrolysis batches.

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MATERIALS AND METHODS

Candida rugosa lipase (890 units mg^{-1}) from Sigma Chemical Co. (St. Louis, MO) was used for experiments without further purification. Refined Malaysian palm oil (Vital Vrbas, Yugoslavia) with a saponification value of 199.5 was used as the substrate for lipase hydrolysis. Isooctane of p.a. grade was purchased from Merck (Darmstadt, Germany) and used as the organic solvent.

Experimental setup

The experimental setup (Fig. 1A) consisted of a hollow fiber reactor, oil and buffer reservoirs, two pumps for recirculation of the oil and water phases and an external water jacket. Capillary dialysis modules (model E2) purchased from INEX–Hemofarm (Vrsac, Yugoslavia) were used as hollow fiber reactors (Fig. 1B). The total membrane area was 1.0 m^2. The fibers were made of *Cuprophane* with a narrow molecular weight cut–off (MW CO) of 5000 Da. In each batch experiment, 140 cm^3 of the oil phase was recirculated through the lumen side and 200 cm^3 of the aqueous phase was recirculated through the shell side of the reactor. Both phases were recirculated cocurrently upwards through the vertically oriented reactor by peristaltic pumps. The reactor had an external jacket recirculated by hot water to maintain isothermal conditions. All hydrolysis reactions were conducted at 30±1°C and pH of 7.0±0.05. Reactor active lumen volume was determined as 64 cm^3 and was recirculated at a flowrate of 16 cm^3 min^{-1}, thus giving a reactor residence time of 0.067 h.

Lipase immobilization

Preparation of the lipase solution and immobilization of the lipase were previously described (Knezevic et al., 2004). The reactor lumen was recirculated with the aqueous lipase solution and subsequently filled with the organic solvent, which resulted in lipase immobilization on the inner side of fibers. The amount of immobilized lipase was determined as a difference between the protein content

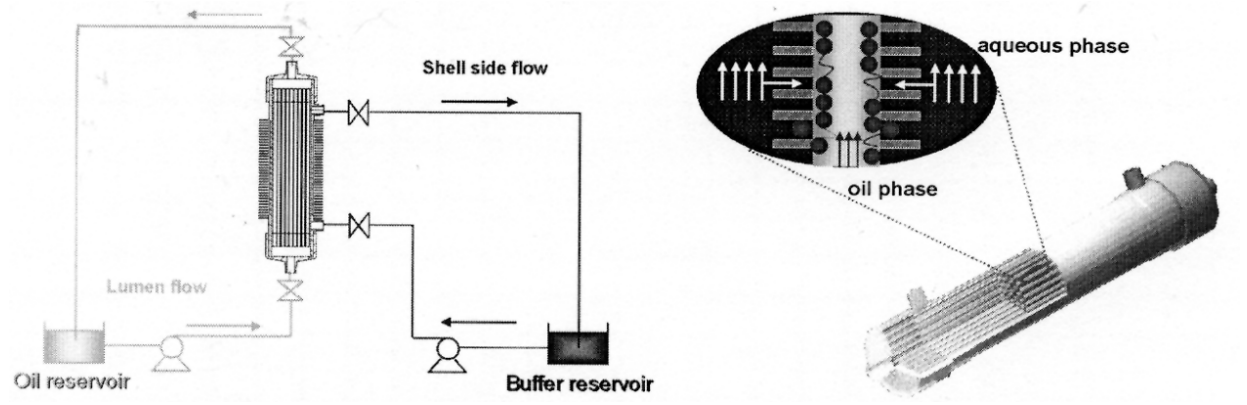

Figure 1. A) Experimental setup for lipase–catalyzed hydrolysis of palm oil; B) Hollow fiber module

of the lipase solutions before and after the immobilization procedure.

Palm oil hydrolysis in the membrane reactor

The oil phase (140 cm^3, palm oil solution in isooctane) was recirculated through the reactor lumen side at 16 cm^3 min^{-1} whereas the aqueous phase (200 cm^3, 0.5 M phosphate buffer pH 7) was recirculated at 9 cm^3 min^{-1} through the shell side. Aliquots of the oil phase were taken at timed intervals for free fatty acids analysis (Knezevic et al., 1998). After each hydrolysis batch, the oil and the aqueous phases were withdrawn and exchanged with equal volumes of fresh solutions. In studies of stability of immobilized lipase, 20 successive batches of palm oil hydrolysis were performed in the same membrane reactor. Each batch lasted for 7–8 h after which the substrate was exchanged with the fresh one.

Palm oil hydrolysis in the microemulsion system

The enzyme reaction in the microemulsion system was assayed as described previously (Knezevic et al., 1998). The bottles of 100 cm^3 were filled with 12 cm^3 of 160 mmol dm^{-3} soya lecithin solution in isooctane containing the substrate at various concentrations (0.059–0.326 mol dm^{-3}). The hydrolysis reaction was initiated by adding 500 µl lipase solution in 0.5 M phosphate buffer pH 7 to the substrate reaction mixture, followed by vortexing until the solution become homogenous. Reactions were carried out at 30°C using a shaking water bath at 130 strokes per min.

Mathematical Modelling

In order to interpret and analyze the obtained experimental results, we have applied the kinetic model previously derived for palm oil hydrolysis in a microemulsion system (Knezevic et al., 1998). We have assumed that the reaction carried out by the immobilized lipase in the membrane reactor follows the same kinetics as that determined for free lipase in the microemulsion system. In addition, we have supposed plug flow in the lumen zone of the reactor and ideal mixing in the oil reservoir. The experimental system could thus be represented by a two–compartment model consisting of a plug flow reactor connected in series with an ideally mixed vessel.

Palm oil hydrolysis in the membrane reactor was modeled as the one–substrate two–products first–order reversible reaction with no influence of water concentration. The mechanism of the reaction may be represented as follows:

$$S + H_2O \underset{k_2}{\overset{k_1}{\rightleftarrows}} P_1 + P_2$$

where S is the ester bond which can be attacked by lipase, P_1 and P_2 are products (free fatty acids and glycerol residues) and k_1 and k_2 are rate constants for ester bond decomposition and formation, respectively. At a steady state, the substrate concentration at the membrane reactor outlet, C_2, can be then expressed as a function of the inlet, C_1, and equilibrium, C_e, substrate concentrations as (Knezevic et al., 1998):

$$C_2 = \frac{C_1 (C_e + C_1 M)}{C_1 + C_e M} \qquad (1)$$

where M is a constant defined as: $M = \exp\left[\frac{k_1\tau(X_e - 2)}{X_e}\right]$, X_e is the equilibrium degree of hydrolysis and τ is the mean reactor residence time.

Mass balance for the oil reservoir where no reaction takes place can be written as:

$$\upsilon C_2 - \upsilon C_1 = V\frac{dC_1}{dt} \qquad (2)$$

where υ is the oil phase volumetric flowrate, V is the reservoir volume, and t is time.

Substituting the Eq. (1) into the Eq. (2) results in a first order differential equation, which can be integrated and solved for the time, t, required to reduce the substrate concentration from the initial concentration, C_o, to a concentration, C, according to:

$$t = \frac{\tau}{M-1}\left[(1+M)\ln\frac{C-C_e}{C_o-C} - M\ln\frac{C}{C_o}\right] \quad (3)$$

In the proposed kinetic model, adjustable parameters are the rate constant, k_1, and the equilibrium degree of hydrolysis, X_e, which were determined by least–squares fits to the experimental data.

RESULTS AND DISCUSSION

Lipase immobilization

In the experimental reactor, the lipase from *Candida rugosa* was immobilized on the hydrophilic Cuprophane hollow fiber membrane. Immobilization technique used in this work was based on non–covalent interactions of water–soluble lipase and hydrophilic hollow fibers. The amount of crude lipase immobilized onto the hollow fibers was 155 ± 5 mg m^{-2} membrane with approximately 40% mass yield.

Palm oil hydrolysis by immobilized lipase: kinetic model

Figs. 2 and 3 show a reaction kinetic profile at different initial substrate concentrations in microemulsion and membrane reactor systems, respectively. Points on the graph are average experimental data and the solid lines represent the best fits of the theoretical model predictions for different substrate concentrations. Results show that equilibrium degree of hydrolysis generally decreased as the initial substrate concentration was increased. The highest degree of hydrolysis of approximately 99% was achieved for the lowest initial substrate concentration in the membrane reactor (Table 1). Nevertheless, the reaction rate with immobilized lipase was lower than that of free lipase as implied by the obtained values of reaction rate constants in the two systems. On the other hand, in the membrane reactor system, the continual removal of the glycerol

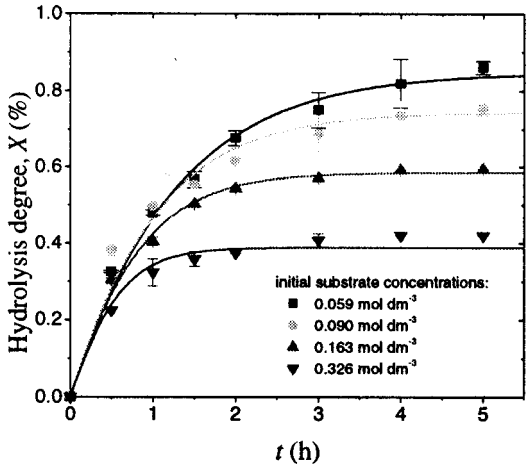

Figure 2. Hydrolysis degree as a function of reaction time at different substrate concentrations in the microemulsion system

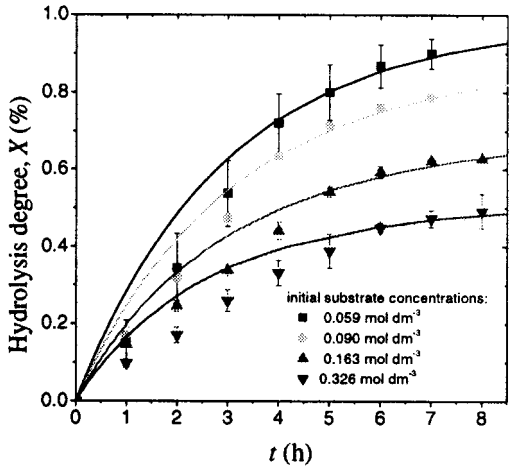

Figure 3. Hydrolysis degree as a function of reaction time at different substrate concentrations in the membrane reactor system

from the membrane in the aqueous phase reduced the back reaction rates and allowed higher conversions.

The experimental results obtained in the microemulsion system agreed well with the kinetic model in the investigated range of initial substrate concentrations (average STD was 1.61%). On the other hand, the applied model somewhat overestimated the reaction rate at the beginning of hydrolysis in the membrane reactor (Fig. 3). Overally, the model predicted the reactor performance reasonably well (average STD was 11.54%). This evidence supports the contention that the nature of immobilized lipase in the membrane reactor is similar to the nature of the free enzyme in microemulsion. Based on the average values of the rate constants at 30°C (0.64 h^{-1} and 0.28 h^{-1} for the free and the immobilized enzyme, respectively) it can be stated that the immobilized enzyme retained around 44% activity of the free enzyme.

Stability of the immobilized lipase was assessed in 20 successive batches of palm oil hydrolysis performed in the same membrane system. Observed specific rates in the first four hydrolysis runs were only slightly higher than those obtained after 20 cycles (137 h) despite the considerable loss of the immobilized enzyme. The overall decrease in activity of the immobilized lipase was around 15%.

Table 1. Summary of kinetic parameters determined in the microemulsion and the membrane system

C_0 (mol dm^{-3})	Microemulsion system			Membrane system		
	X_e	k_1 (h^{-1})	STD (%)	X_e	k_1 (h^{-1})	STD (%)
0.059	0.836	0.648	2.655	0.990	0.339	11.019
0.090	0.741	0.761	0.902	0.910	0.293	8.696
0.163	0.585	0.650	0.601	0.703	0.240	10.639
0.326	0.389	0.508	2.269	0.518	0.260	15.822

STD (%) is the average standard deviations between the predict values and experimental data

CONCLUSIONS

In this work, the feasibility of lipase immobilization in a hydrophilic hollow fiber membrane reactor for oil hydrolysis has been demonstrated. A simple immobilization technique was applied resulting in about 155 mg m^{-2} of immobilized lipase at approximately 40% mass yield. The reaction in the membrane reactor was well approximated by one–substrate first–order reversible kinetics, previously established for a microemulsion reactor, implying the same reaction mechanism in the two systems. At the enzyme load employed in this study, loss of activity due to enzyme desorption had little effect on reactor stability and oil hydrolysis. The membrane reactor operated for up to 137 h with no significant loss in productivity.

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