

Mass Transfer Effects in Immobilized Lipase Packed Bed Reactor during the Hydrolysis of Rice Bran Oil

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External mass transfer effects during the hydrolysis of rice bran oil using immobilized lipase enzyme were studied in a differential recirculation reactor. On set of external mass transfer limitations were found to be responsible for the optimum activity with respect to the enzyme loading. Particle dimension influences global reaction rate more at lower flow rates than at higher flow rates. Experiments performed in continuous immobilized lipase packed bed reactor to study the influence of substrate concentration and flow rate on fractional hydrolysis of rice bran oil showed that the apparent kinetic constants vary with flow rates.

Keywords:

Rice bran oil, differential reactor, optimum enzyme loading, mass transfer effects, packed bed reactor, kinetic constants.

Introduction

Fatty acids and glycerol are essential chemicals in the oleochemical industry. Presently these chemicals are produced from fats using a continuous high-pressure uncatalyzed counter-current method. Enzymatic splitting of fats is gaining importance, as it is an energy saving process that can be carried out at ambient temperature and atmospheric pressure. Hydrolysis of olive oil, tallow and palm oil using lipase has been reported by many authors.^{1–7}

The mass transfer effects influence the performance of the immobilized enzyme reactor. Mass transfer effects are limited by internal or pore diffusional resistance and external mass transfer resistance. The external mass transfer effects on the performance of different immobilized reaction systems were studied by various authors.^{8–12} In our present study, since the enzyme was immobilized on acid-washed glass beads with negligible porosity, the internal diffusion effects were negligible and external mass transfer effects during the hydrolysis of rice bran oil were studied in a differential recirculation packed bed reactor. The optimum enzyme loading that determines the maximum activity under the external mass transfer limitation was estimated and influence of superficial velocity on global reaction rate was analysed, for two different sizes of enzyme immobilized glass beads. Effects of substrate mass concentration and fluid flow rate on fractional hydrolysis were also studied in a continuous immobilized packed bed reactor, and influence

of flow rate on apparent kinetic constants was analysed.

Materials and methods

Materials

Crude lipase enzyme preparation from *Candida rugosa* (formerly *Candida cylindracea* EC 3.1.1.3, 285 U mg⁻¹) was obtained from Sigma Chemicals Co.(St.Louis,MO,USA). This preparation was used without further purification to prepare the immobilized enzyme. Acid washed glass spherical beads, 1 mm and 2 mm diameter (Sigma) were used as enzyme support material for immobilization. 3-Aminopropyltriethoxysilane used for generating functional groups on glass beads was obtained from Acros organics (NJ, USA). All chemicals used were reagent grade and were obtained from Nice Chemicals (Cochin, India). Rice bran oil (saponification value = 180, iodine value = 90, $w_{\text{FFA}} = 0.3\%$) was obtained from Sri Jayasakthi Rice&Oil mills (Salem, India).

Immobilization of lipase enzyme

Lipase enzyme from *Candida rugosa* was immobilized on acid washed activated glass beads based on the method developed by Wu and Weng,¹³ and described in detail in our earlier paper.¹⁴ The enzyme loadings of 0.74–3.25 mg g⁻¹ of support were obtained, using different fractions of enzyme attachment solutions.

Analytical methods

The activity of lipase is described in terms of lipase units (U). One unit (U) of lipase is defined as the amount of enzyme required to produce one μmol of free fatty acid in one minute under assay conditions.

Free fatty acids liberated were measured by spectrophotometric method as described by *Kwon and Rhee*.¹⁵ Initial rate was measured by finding the initial slope of the plot of μmol of free fatty acids produced versus time. Ratio of this initial rate and mass of the enzyme gives the activity.

Protein measurements were performed according to a modified Lowry procedure¹⁶ Fractional hydrolysis or conversion (X) was determined using the formula:

$$\text{Fractional hydrolysis} = \frac{\text{amount of fatty acids liberated}}{[\text{saponification value} / (3 \times 56.1)][1000 \times \text{mass of oil}]}$$

Experimental

Packed bed reactor

Packed bed reactor was a glass column, 20 cm long and 2.54 cm internal diameter, having a jacket through which water at desired temperature was circulated. Enzyme immobilized glass beads were packed in between glass wool in the column and the headspace was filled with larger, 3 mm plain glass beads.

Study of external mass transfer effects in differential recirculation reactor

Fig. 1 shows the differential reactor setup used in the study of external mass transfer effects. 250 mL of each rice bran oil and water (phosphate buffer pH 7.2) were mixed to form a fine emulsion at predetermined temperature. This emulsion was pumped using peristaltic pump (Muropye scientific company, India, 10 mL hr⁻¹–2000 mL hr⁻¹ flow rate range) at 8 mL min⁻¹ through the bed packed with 92 g of immobilized enzyme glass beads and a constant temperature of 42 °C was maintained using high precision circulating water bath (Muropye scientific company, India, ± 0.2 °C accuracy). 200 μL of emulsion was sampled from the mixing tank at predetermined time intervals and the amount of free fatty acids formed was estimated. The experiments were conducted using different enzyme loadings of 1 mm and 2 mm di-

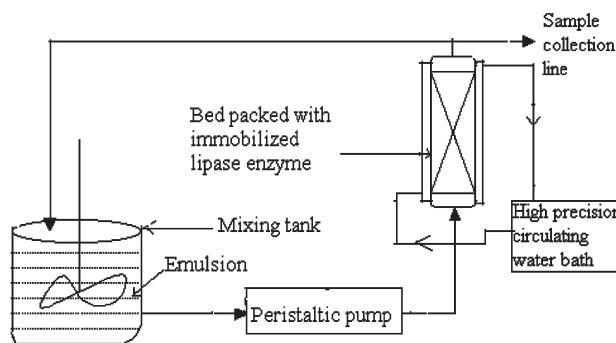


Fig. 1 – Differential recirculation reactor set up for the study of external mass transfer effects

ameter beads, initial reaction rates and activities were determined, and the optimum enzyme loading beads were identified. Samples were also taken from the reactor outlet to check that the conversions per pass were not too high. The plots of conversion versus time of the samples from reactor outlet and the mixing tank were perfectly parallel straight lines, with maximum variation being nearly 10 % at the lowest flow rates.

In a second set of experiments, carried out to study the influence of external mass transfer on global reaction rate through the layer of stagnant film, optimum enzyme loading beads of 1 mm, 2 mm diameter were used as the bed packing material, and fluid flow rate was varied from 4 mL min⁻¹–20 mL min⁻¹.

Experiments in continuous packed bed reactor

The experimental setup is shown in Fig. 2. The column used is the same as that used in the differ-

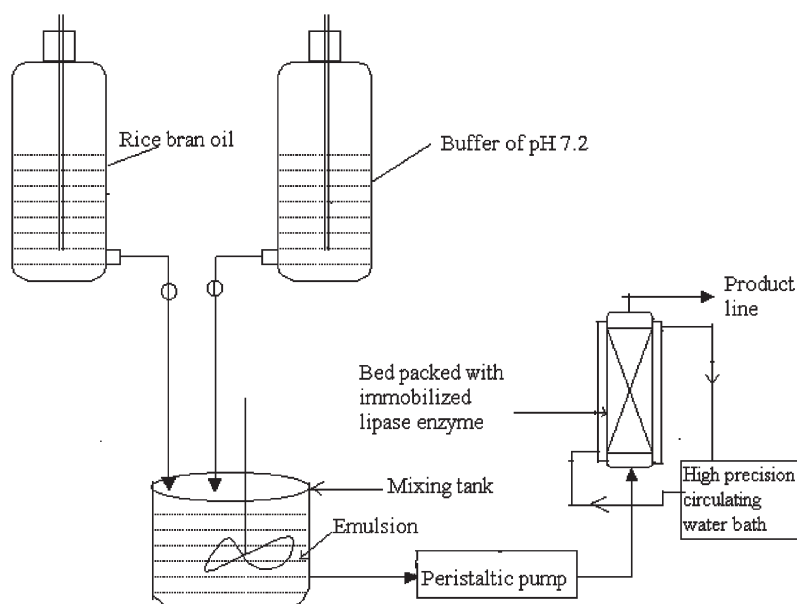


Fig. 2 – Schematic diagram of immobilized lipase continuous packed bed reactor for the hydrolysis of rice bran oil

entail reactor experiments. The column was packed to a height of 14 cm with 92 grams, 2 mm diameter and 2.15 mg g⁻¹ optimal enzyme loading beads. Initially proper ratio of Rice bran oil and water (phosphate buffer of pH 7.2) were taken in to one liter capacity mixing tank at predetermined temperature. Then the substrate was continuously prepared by mixing the reactants in the same ratio from their respective reservoirs. This substrate was pumped at constant flow rate through the bed. A constant temperature of 42 °C was maintained. The stream that contains products and unreacted substrates was continuously collected from the top of the packed bed. Samples of 200 μL were collected from the reactor out let and free fatty acid content at steady state was estimated. The fractional hydrolysis data were generated for different substrate concentrations and flow rates. The flow rates were chosen such that emulsion was stable.

Theoretical

A model equation for immobilized enzyme packed bed reactor as suggested by O’Neill et al¹⁷ is

$$X[S_0] = K_m^1 \ln(1-X) + \gamma_{max}^1 (h/v_s) \quad (1)$$

The values of apparent kinetic constants can be obtained from slope and intercept of the plot of $X[S_0]$ versus $\ln[1-X]$.

The colburn-type correlation¹⁸ was applied to represent the mass transfer coefficient, k_L ; i.e

$$j_D = C(Re)^{-P} \quad (2)$$

where j_D is the mass transfer j -factor defined as

$$j_D = \left[\frac{k_L}{v_s} \right] \cdot \left[\frac{\mu}{\rho D_f} \right]^{2/3} \quad (3)$$

C and P are the correlation coefficients. Then for the fixed fluid properties

$$k_L \propto \frac{v_s^{1-P}}{d_p^P} \quad (4)$$

Park et al.⁹ had estimated correlation coefficient, P for various immobilized enzyme reaction systems in a packed bed reactor which for most liquid phase systems were close to 0.5. The difference in film resistance ($\Delta R_{2,1}$) for two different bed particle diameter can therefore be expressed as

$$\Delta R_{2,1} \propto \frac{1}{v_s(1-P)} [d_{p2}^P - d_{p1}^P] \quad (5)$$

Results and discussions

Effect of enzyme loading on immobilized lipase activity

The effect of enzyme loading on activity for 1 mm and 2 mm diameter particles are shown in Fig. 3. Each dimension has an optimum enzyme loading at which activity is maximized. Activity increased up to the enzyme loading of 2.15 mg g⁻¹ bead, for both dimension of beads, and thereafter decreased. The decrease in activity may be due to the denaturation of lipase or onset of external mass transfer effects. We had found¹⁹ negligible amount of denaturation for this particular immobilized enzyme at the same working temperature and pH with a loss of only 10 % activity in 24 h time duration. Hence, decrease of activity must be due to external mass transfer limitations. In order to verify this, reaction rate instead of activity was plotted against enzyme loading in Fig. 4. The rate

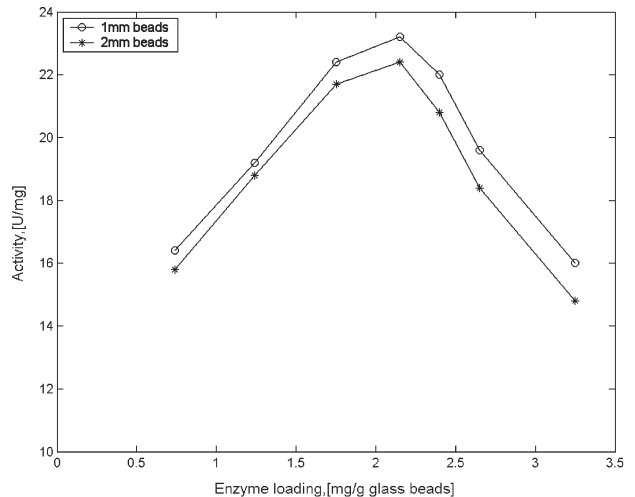


Fig. 3 – Effect of enzyme loading on activity of immobilized lipase. 250 mL each = 50% rice bran oil and water were used for the reaction and flow rate was 8 mL min⁻¹

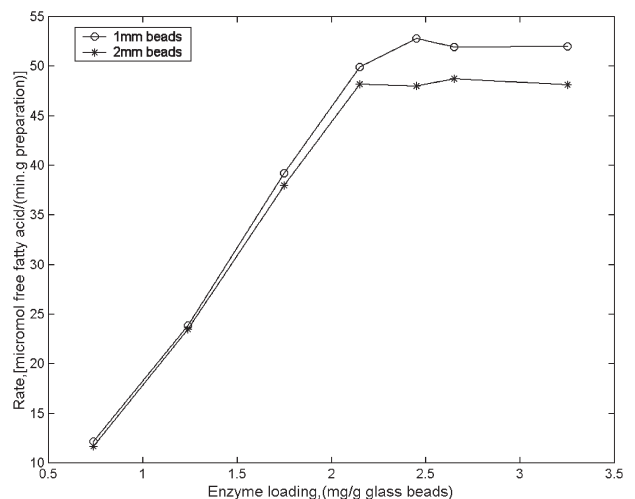


Fig. 4 – Another way of representation of the data in Fig. 3. Reaction rate was used instead of activity

increased with enzyme loading reached a maximum, and thereafter remained constant. This represents the maximum rate at which the substrate was transported from bulk solution to the interface and reacted. It was also observed that smaller the support particle diameter higher the maximum reaction rate. Interfacial area for mass transfer per unit volume of bed is greater for smaller particles, which increases the mass transfer rate and hence reaction rate.

Effect of superficial velocity on initial reaction rate

This effect was shown in the Fig. 5 for two different dimensions of support particles. Reaction rate increased with flow rate and reached a constant plateau. As the fluid flow rate increases the thickness of the stagnant layer around the particle decreases and the external film resistance for mass transfer decreases. Consequently, global reaction rate shifts from mass transfer limited region to chemical reaction limited region. The difference in reaction rate for 1 mm and 2 mm diameter of bed particles is greater at lesser flow rates than at higher flow rates. This is in accordance with eqn5, which indicates that the difference in film resistance decreases with increase in superficial velocity.

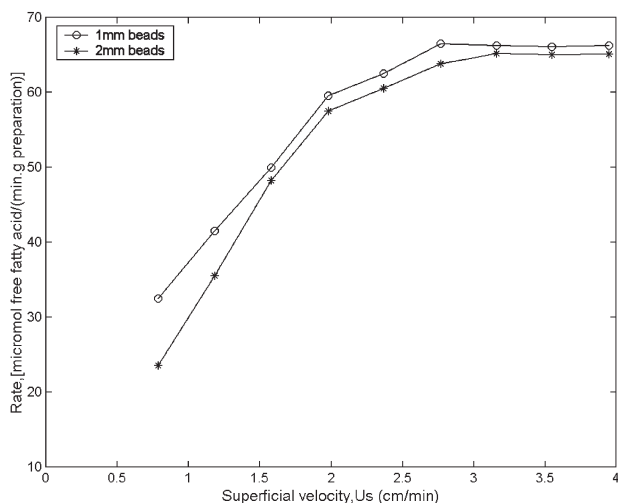


Fig. 5 – Effect of superficial velocity on initial reaction rate. 250 mL each = 50% oil and water, 2.15 mg g⁻¹ bead loading of 1 mm and 2 mm immobilized beads were used as the bed material

Effect of flow rate and substrate mass concentration on fractional hydrolysis

Based on the experimental data obtained from continuous packed bed reactor, the fractional hydrolysis was plotted against the flow rate and shown in Fig. 6. The fractional hydrolysis was found to decrease with increase in flow rate. This is due to the decrease of mean residence time of the reacting

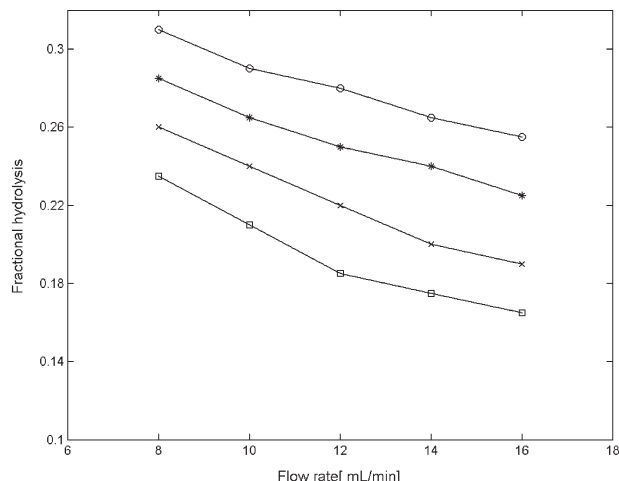


Fig. 6 – Effect of fluid flow rate on fractional hydrolysis of rice bran oil in a continuous packed bed reactor at different initial substrate concentrations. The initial substrate concentrations were: (○)0.195; (*)0.292; (×)0.389; (□)0.487 mol l⁻¹. 92 grams of 2 mm diameter and enzyme loading of 2.15 mg g⁻¹ bead were used.

fluid in the reactor. It was also noticed that at a particular flow rate the fractional hydrolysis increased with decrease in substrate mass concentration. These experimental findings are justified by the theoretical model proposed by Marrazzo et al.²⁰ for Michaelis-Menten reaction kinetics, which predicts the decrease of conversion with increase in substrate concentration for the zero-order reaction region.

Effect of flow rate on apparent kinetic constants

The values of K_m^1 and γ_{max}^1 were determined by plotting $X[S_0]$ versus $\ln[1-X]$ with flow rate as the variable parameter as shown in Fig. 7. From the

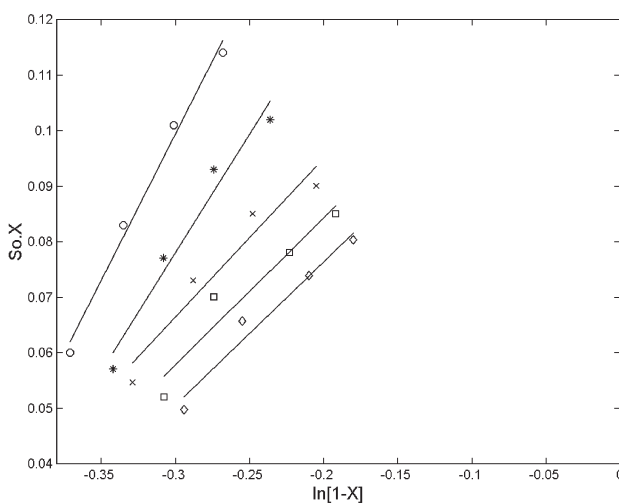


Fig. 7 – Relationship between $[S_0] \cdot X$ versus $\ln[1-X]$ at different fluid flow rates in the continuous packed bed reactor. The flow rates used were: (○)8; (*)10; (×)12; (□)14; (◇)16 mL min⁻¹

slope and intercept of the different straight lines the values of K_m^1 and γ_{\max}^1 , were calculated. It is seen that with increase in superficial velocity K_m^1 decreased and γ_{\max}^1 is almost constant as shown in Fig. 8. Lilly et al.²¹ and Kobayashi et al.²² had made similar observations for ficin chemically bound to carboxymethyl cellulose and immobilized invertase attached to ion-exchange resins in column reactor. However, many authors also reported contradictory behaviour of the kinetic constants. While some authors¹² had reported that K_m^1 decreased and γ_{\max}^1 increased with an increase in the superficial velocity for immobilized glucose isomerase in packed bed reactor system, some others^{23,24} reported that K_m^1 remained constant but γ_{\max}^1 increased with flow rate. This mode of change in the value of apparent K_m^1 and γ_{\max}^1 in the present work indicates that the rate of hydrolysis is governed not only by the chemical reaction but also by mass transfer considerations.

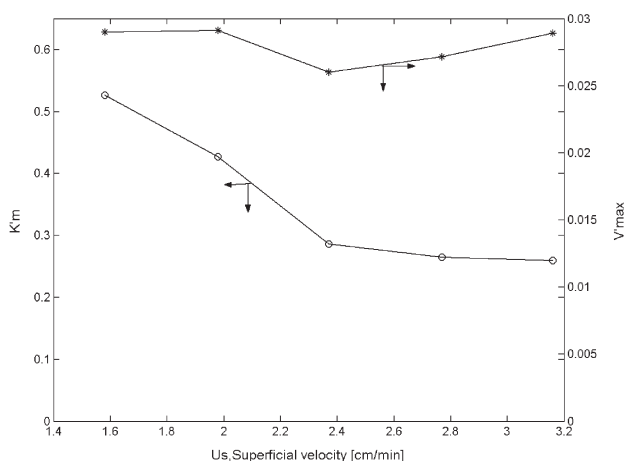


Fig. 8 – Effect of superficial velocity (v_s) on apparent kinetic constants, K_m^1 and γ_{\max}^1

Concluding remarks

The conversions those obtained during the continuous operation of packed bed reactor were quite low. Maximum conversion of about 31 % was achieved when minimum flow rate was used in the continuous operation. However, higher conversion can be obtained using recycling of the reacting fluid.

For the reaction system studied on set of mass transfer effects seemed to be responsible for the optimum enzyme loading. Optimization of enzyme loading is very important in enzyme-catalyzed process. An immobilized enzyme operating under the conditions of mass transfer limitation will show decrease of apparent activity. In the present work higher apparent activity was observed with smaller bed particles under mass transfer limitations. But

the smaller particles will contribute higher-pressure drops in the packed bed. So, an economically optimal particle diameter should then be found that compromises the pressure drop and apparent activity. It was observed that external film resistance decreased with increase in fluid flow rates. However, fractional hydrolysis decreased with increase in fluid flow rate or decrease in mean residence time in the continuous operation. Variation of kinetic constants with flow rate indicates that mass transfer effects influenced the global reaction rate.

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Abbreviations

- D_f – Diffusivity of substrate, $m^2 s^{-1}$
- d_p – Particle diameter, m
- h – Height of the bed packed with immobilized lipase, m
- k_L – Mass transfer coefficient, $m s^{-1}$
- K_m^1 – Apparent Michaelis–Menten constant, $kmol m^{-3}$
- m – mass, g
- Re – Reynolds number $Re = \frac{d_p v_s \rho}{\mu}$, –
- $[S_0]$ – Initial substrate concentration, $kmol m^{-3}$
- v_s – Superficial velocity, $m^3 s^{-1} m^{-2}$, $m s^{-1}$
- γ_{\max}^1 – Apparent maximum reaction rate, $kmol m^{-3} s^{-1}$
- μ – Viscosity, $kg m^{-1} s^{-1}$
- ρ – Density of substrate, $kg m^{-3}$
- n – amount, μmol

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