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

V. R. C. Murty*, J. Bhat, and P. K. A. Muniswaran
Department of Chemical Engineering, Manipal Institute of Technology
A Constituent Institution of Manipal Academy of Higher Education
(Deemed University), Manipal-576104, India.
Tel.: (91–0820–2573660), Fax: 2571071, E-mail: vytlarama@yahoo.com

Original scientific paper
Received: December 9, 2003
Approved: October 1, 2004

External mass transfer effects were studied in immobilized lipase packed bed reactor during the hydrolysis of rice bran oil. Global mass transfer coefficients were estimated at different fluid flow rates and feed substrate concentrations. A relation between global mass transfer coefficient and superficial velocity was established at given feed substrate concentration. The reaction data were represented in dimensionless Eadie-Hofstee plot, which indicated the extent of film diffusion in packed bed reactor. Film effectiveness factor was calculated experimentally in the packed bed reactor and compared with the theoretically predicted values. Very good agreement was observed between computer simulation using plug flow model with derived mass transfer correlation and the experimental results obtained from immobilized lipase packed bed reactor operation.

Key words:
External mass transfer effects, effectiveness factor, global mass transfer coefficients, immobilized lipase, packed bed reactor and rice bran oil

Introduction

The mass transfer effects of substrates and products influence reaction rates of immobilized enzymes in packed bed reactor systems, and many authors have investigated mass transfer effects for enzymes bound to solid supports.[1–8] Internal or pore diffusion and external mass transfer limit the mass transfer effects. In the present study, as the enzyme was immobilized on acid-washed glass beads with negligible porosity, the internal diffusion effects were negligible, and hence only external diffusion effects are discussed.

Mass transfer studies were conducted in packed bed reactor and data were reported in our earlier paper.[8] The objectives of this paper are the estimation of mass transfer coefficients, representation of the reaction data in dimensionless Eadie-Hofstee plot, which identify the diffusion and reaction limiting regions, determination of effectiveness factor theoretically and experimentally, and comparison of theoretically predicted conversion with experimental values during hydrolysis of rice bran oil in immobilized lipase packed bed reactor.

Materials and methods

Materials

A lipase (EC 3.1.1.3, 285 U mg^{-1}, Sigma) preparation from *Candida rugosa* (previously *Candida cylindracea*) was used without further purification to prepare the immobilized enzyme. Acid washed glass beads spherical, 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, FFA = 0.3 %) was obtained from Sri Jayasakthi Rice&Oil mills (Salem, India).

Immobilization of lipase enzyme

Lipase enzyme was immobilized on acid washed activated glass beads based on the method developed by *Wu* and *Weng*,[9] and described in detail in our earlier paper.[10]

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 micromole 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*.[11] Protein measurements were performed according to a modified Lowry procedure.[12]

Fractional hydrolysis or conversion (X) was determined using the formula:[13]

$$\text{Fractional hydrolysis } (X) = \frac{\mu\text{mol of fatty acids liberated } (n)}{[\text{saponification value}/(3\times 56.1)]\ (M_{\text{NaOH}})\ [1000\times \text{g of oil}]\ (m)}$$

Theoretical

Considering a tubular reactor of height h, material balance at steady state for the substrate gives the differential equation:

$$0 = D\ \mathrm{d}^2c_S/\mathrm{d}x^2 - v_S\ \mathrm{d}c_S\ /\mathrm{d}x - (k_l\, a)(c_S - c_{S*}) \quad (1)$$

First term in the right hand side represent the axial dispersion of substrate. Assuming plug flow and neglecting mixing in axial direction eqn. (1) can be written as

$$\mathrm{d}c_S\ /\mathrm{d}x = (-\ k_l\, a\ /\ v_S)\ (c_S - c_{S*}) \quad (2)$$

At $x = 0$, $c_S = c_{S0}$ and $x = h$, $c_S = c_{Sf}$

$$c_S = c_{S0}\,(1 - X) \quad (3)$$

At steady state mass transfer rate is equal to the reaction rate (γ), which for Michaelis-Menten kinetics

$$k_l\, a\ (c_S - c_{S*}) = \gamma^1{}_{\max} \cdot c_{S*}\ /(K^1{}_m + c_{S*}) \quad (4)$$

From Eqn. (4)

$$c_{S^*} = \frac{-K + \sqrt{K^2 + 4K'_m c_S}}{2} \quad (5)$$

Where K is a function of substrate concentration

$$K(\mathrm{S}) = K^1_m + \frac{\gamma'_{\max}}{k_1 a} - c_S \quad (6)$$

Integrating eqn. (2);

$$h = \left(\frac{-v_S}{k_1 a}\right)\int_{c_{S_0}}^{c_{Sf}} \frac{1}{(c_S - c_{S^*})}\,\mathrm{d}c_S \quad (7)$$

Eqn. (7) is used for estimation of global mass transfer coefficient on trial and error basis.

If the Colburn-type correlation $j_D = C(Re)^{-P}$, is applied to represent the mass transfer coefficient, one can obtain $k_l\, a$ as a function of superficial velocity.

$$k_1 = C\left[\frac{\rho\, v_S d_p}{\mu}\right]^{-P} \cdot v_S \cdot \left[\frac{\mu}{\rho D_f}\right]^{-2/3} \quad (8)$$

$$k_l\, a = A\ v_S{}^{1-P} = A.\ v_S{}^q \quad (9)$$

Where A and q are the constants.

By fitting the experimentally determined $k_1\, a$ values in eqn. (9) the constants A and q can be determined.

Eqn. (4) expressed in dimensionless form by defining the following dimensionless quantities:

Dimensionless bulk substrate concentration, $c_{Bb} = c_S\ /\ K^1{}_m$

Dimensionless interfacial substrate concentration, $c_{B*} = c_{S*}\ /\ K^1{}_m$

Damköhler number, $Da = \gamma^1{}_{\max}\ /(K^1{}_m\ k_l\, a)$

At $x = 0$, $c_{Bb} = c_{B0} = c_{S0}\ /\ K^1{}_m$ and at $x = h$, $c_{Bb} = c_{Bf} = c_{Sf}\ /\ K^1{}_m$

Eqn. (4) becomes

$$(c_{Bb} - c_{B*}) = \mathrm{Da} \cdot c_{B*}\ /(1 + c_{B*}) \quad (10)$$

Dimensionless rate,

$$R = \gamma/\ \gamma^1{}_{\max} = c_{B*}/(1 + c_{B*}) = f(c_{Bb}, \mathrm{Da}) \quad (11)$$

The reaction data can be represented in dimensionless Eadie-Hofstee plot in which R is plotted against R/c_{Bb} with Damköhler number as the parameter.

From eqn. (10) the interfacial concentration in dimensionless form can be obtained as

$$c_{B^*} = \frac{-(1 + Da - c_{Bb}) + \sqrt{(1 + Da - c_{Bb})^2 + 4c_{Bb}}}{2} \quad (12)$$

$$\text{Film effectiveness factor } (\eta_f) = \frac{\text{Observed reaction rate with film resistance}}{\text{Reaction rate unhindered by film resistance}}$$

$$\eta_f = c_{B*}\ (1 + c_{Bb})\ /\ c_{Bb.}(1 + c_{B*}) \quad (13)$$

Eqns. (12) and (13) are used for prediction of film effectiveness factor in the packed bed reactor. The interfacial concentrations and bulk substrate concentrations vary in the axial direction over the length of the packed bed reactor and the average interfacial concentration is given by

$$c_{S^*,\,\text{avg.}} = \frac{-\int_{c_{S_0}}^{c_{Sf}} c_{S^*}\mathrm{d}c_S}{(c_{S_0} - c_{Sf})} \quad (14)$$

Since the packed bed reactor is acting as a differential reactor, average interfacial concentration calculated by eqn (14) is almost equal to the arithmetic average.

Average bulk substrate concentration

$$\bar{c}_B = (c_{S_0} + c_{Sf})/2 \quad (15)$$

In our earlier work,[8] in order to confirm the differential reactor, samples were taken from the reac-

tor outlet and mixing tank 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.

These average concentrations calculated from eqn. (14) were used for evaluating the average effectiveness factor in the packed bed reactor.

Eqn. (2) can be expressed in terms of conversion as

$$dX/dz = k_l\, a\, \tau\, [(1 - X) - c_{S*}/c_{S0}] \qquad (16)$$

Where $z = x/h$, $\tau = h/v_S$.

Eqn. (5) can be expressed as

$$\frac{c_{S^*}}{c_{S_0}} = \frac{\dfrac{-K}{c_{S_0}} + \sqrt{\left(\dfrac{K}{c_{S_0}}\right)^2 + \dfrac{4K'_m(1-X)}{c_{S_0}}}}{2} \qquad (17)$$

Substituting eqn. (17) in (16) one gets

$$\frac{dX}{dz} = k_1 a \tau \left[(1-X) + (1/2)\frac{K}{c_{S_0}} - \right.$$

$$\left. -(1/2)\sqrt{\left(\frac{K}{c_{S_0}}\right)^2 + 4K'_m(1-X)}\right] \qquad (18)$$

Where K is a function of conversion

$$K(X) = K^1_m + \frac{\gamma^1_{max}}{k_1 a} - c_{S_0}(1-X) \qquad (19)$$

With the help of eqn (18) and established mass transfer correlation, conversions were predicted at different space times at given substrate concentration using Matlab software.

Experimental

Experiments on hydrolysis of rice bran oil were performed in a column reactor packed with immobilized lipase enzyme at different substrate concentrations and flow rates. The details of the experimental set up: experimental procedure and results were reported in our earlier paper.[8] The experimental data reported in our earlier paper[8] were used for the estimation of mass transfer coefficients in the present paper.

Results and discussion

Global mass transfer coefficients estimation

Effect of fluid flow rate on fraction hydrolysis of rice bran oil in a continuos packed bed reactor at different feed substrate concentrations is showns in Fig. 1.

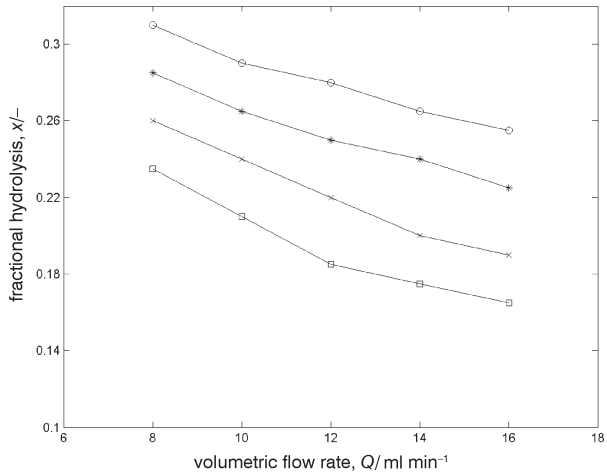


Fig. 1 – *Effect of fluid flow rate on fractional hydrolysis of rice bran oil in a continuous packed bed reactor at different feed substrate concentrations. The feed substrate concentrations were: (o) 0.195; (*) 0.292; (×) 0.389; (□) 0.487 M. 92 grams of 2 mm size and enzyme loading of 2.15 mg/g bead were used. (From Reference 8).*

Global mass transfer coefficients estimated at different velocities and substrate concentrations using eqn (7) employing trial and error technique, are reported in Fig. 2. In this method particular $k_l\, a$ was assumed and R.H.S of eqn (7) was evaluated till it

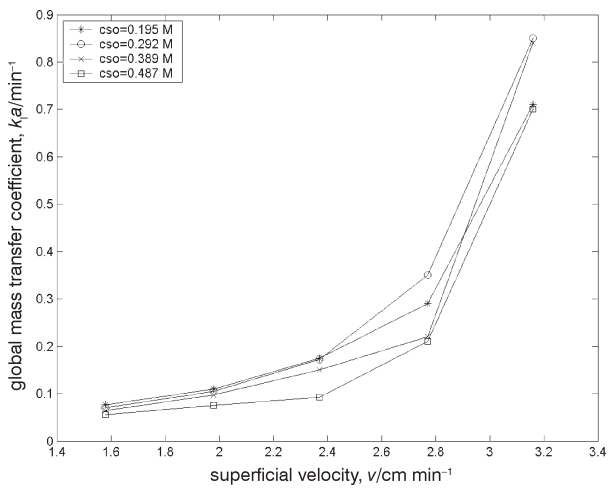
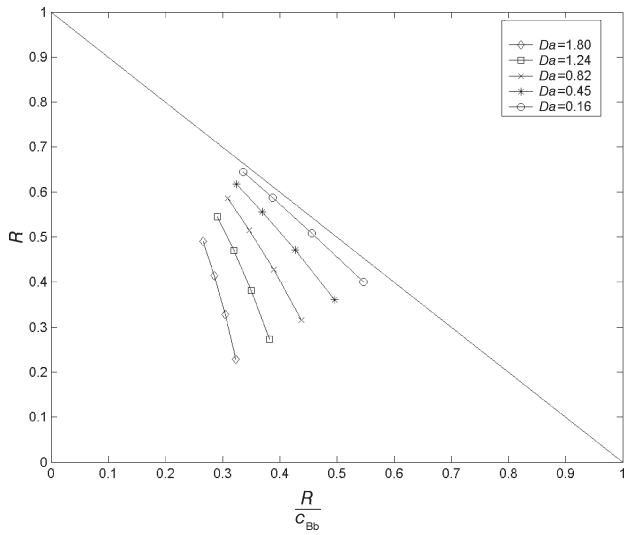


Fig. 2 – *Effect of fluid flow rate on global mass transfer coefficients in packed bed reactor at different initial substrate concentrations. 92 grams of 2mm size and enzyme loading of 2.15 mg/g bead were used. Constants used for calculation of k_l a were: K^1_m=0.2328 mol dm^{-3}, γ^1_{max} = 0.0281 mol dm^{-3} min^{-1} (measured in batch reactor).*

is equal to the bed height of 14 cm. It can be seen that $k_l\,a$ values increase with flow rates and are more sensitive at higher velocities of above 2.8 cm min^{-1}. This may be due to the predomination of eddy diffusion. Feed substrate concentration seems to affect global mass transfer coefficients less significantly compared to the flow rates. The relation between $k_l\,a$ and superficial velocity are obtained from eqn. (9) as $k_l\,a = 0.01548\,v_S^{\,3.054}$ at the substrate concentration of 0.195 mol dm^{-3}. *Lorte* et al[7] had reported q values in the range 0.65 – 1.57 for various immobilized enzyme systems for Michaelis-Menten kinetics in packed bed reactor.

Representation of reaction data in Eadie-Hofstee plot

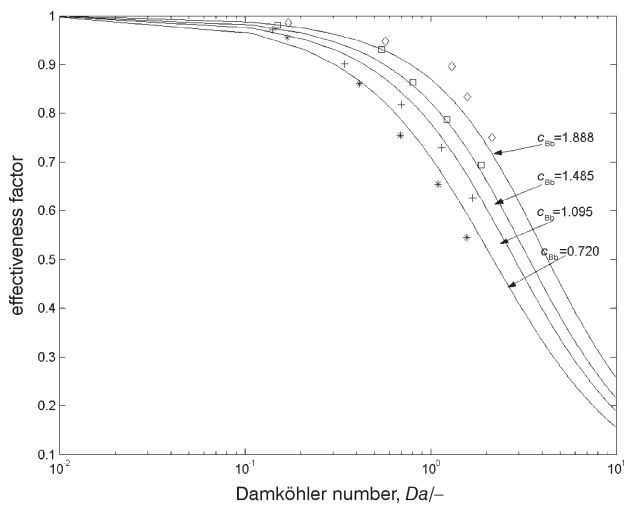
The data were represented in dimensionless Eadie-Hofstee plot with Damköhler number as the parameter in Fig. 3. The dimensionless rate R is evaluated using eqn. (11) in which c_{B*} is the dimensionless interfacial concentration, which varies from bottom to top of the packed bed reactor. The average interfacial concentration was evaluated from eqn. (14) and used for calculation of R at particular velocity or Damköhler number. From Fig. 3 it can be seen that when $k_l\,a$ is very large or Damköhler number is very small, the diffusional rate is very fast compared to the surface reaction and the system is governed by the kinetics. When the $k_l\,a$ is small or Damköhler number is very high then the concentration difference in the film is very steep and the system is governed by diffusion control. From Fig. 3 it is clear that a linear plot at low Damköhler number indicates kinetic control and convex plots at higher values indicate various degree of diffusion control. This dimensionless Eadie-Hofstee plot is very helpful for assessing the type of controlling region and extent of mass transfer difficulty in the hydrolysis reaction of rice bran oil. When $Da = 0.16$ the plot is very close to the diagonal and film resistance is almost negligible. When $Da = 1.8$ the plot is away from the diagonal and almost vertical, which indicates highly diffusion control region.



F i g . 3 – *Dimensionless Eadie-Hofstee plot with Damköhler number as the parameter.*

Plot of Effectiveness factor

The theoretical profiles of h_f vs. *Da,* generated at particular dimensionless substrate concentration using eqns. (12) and (13), are compared with the effectiveness factor determined from experiments in Fig. 4. A good agreement between theoretical and experimental results can be observed from Fig. 4. At all substrate concentrations effectiveness factor decreases as the Damköhler number increases. The bulk substrate concentration seems to have more influence on effectiveness factor at higher Damköhler number than at lower values.



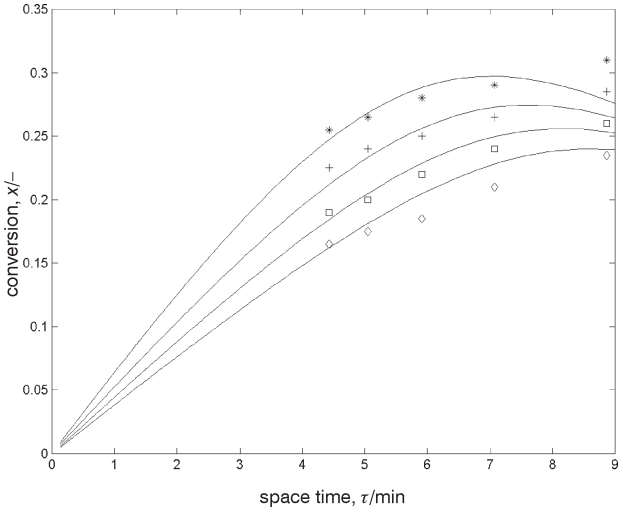
F i g . 4 – *Plot of effectiveness factor vs. Damköhler number with dimensionless bulk substrate concentration as the parameter. The solid lines represent theoretically predicted profiles. The experimental values shown by symbols: The dimensionless bulk substrate concentrations were: (◇) 1.888; (□) 1.485; (+) 1.095; (*) 0.720.*

Prediction of conversion in packed bed reactor

The mass transfer correlation eqn. (9) and the model eqn (18) were used for predicting conversion for different substrate concentrations. The predicted conversion – space time profiles are shown in Fig. 5. The simulation results are in good agreement with the experimental conversion data.

Concluding remarks

The fluid flow rates influenced the mass transfer coefficients more significantly than substrate concentration. The Eadie-Hofstee plot clearly dis-



F i g . 5 – *Comparison of predicted conversions with experimental values at different feed substrate concentrations. The solid lines represent predicted conversions where as the various symbols represents experimental values. The feed substrate concentrations were: (*) 0.195; (+) 0.292; (□) 0.389; (◇) 0.489 mol dm^{-3}*

tinguished the diffusion and reaction control regions. The substrate concentration influenced the effectiveness factor at higher Damköhler number. The reaction rate was dropped by 20 % when dimensionless substrate concentration was equal to 1.888 and dropped by 45 % when substrate concentration is equal to 0.720 due to the film resistance at Damköhler number of 1.8

ACKNOWLEDGEMENT

The authors thank MAHE management for the financial assistance extended to the current research work.

Abbreviations

D – dispersion coefficient, m^2 s^{-1}
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}
$k_l a$ – global mass transfer coefficient, s^{-1}
$K^1{}_m$ – apparent Michaelis –Menten constant, kmol m^{-3}
n – amount, mol
Re – Reynolds number
c_{So} – feed substrate concentration, kmol m^{-3}
c_{S*} – interfacial substrate concentration, kmol m^{-3}
c_{Sf} – substrate concentration at the out let of the reactor, kmol m^{-3}
v_S – superficial velocity, m s^{-1}
$\gamma^1{}_{max}$ – apparent maximum reaction rate, kmol m^{-3} · s^{-1}
μ – viscosity, kg m^{-1} s^{-1}
ρ – density of substrate, kg m^{-3}
t – space time, s
w – mass fraction, %
X – conversion

References

1. *Lee,Y.Y, Fratzke, A.R., Wun, K., Tsao, G. T.,* Biotechnol. Bioeng. **18** (1976) 389.
2. *Kobayashi, T., Moo-Young, M.,* Biotechnol. Bioeng. **13** (1971) 893.
3. *Park, Y. H., Han, M. H., Rhee, H. K.,* J. Chem. Technol. Biotechnol. **34**B (1984) 57.
4. *Lee, S. B., Kim, S. M., Ryu, D. D. Y.,* Biotechnol. Bioeng. **21** (1979) 2023.
5. *Lortie, R.,* J. Chem. Tech. Biotechnol. **60** (1994) 189.
6. *Raul, J. B., Wehtje, E, Patrick, A.,* Biotechnol. Bioeng. **59** (1998) 364.
7. *Lortie, R., Thomas, D.,* Biotechnol. Bioeng. **28** (1986) 1256.
8. *Murty, V. R. C., Bhat, J., Muniswaran, P. K. A.,* Chem. Biochem. Eng. Q. **18** (2004) 177.
9. *Wu, J.Y., Weng, H. S.,* Biotechnol.Bioeng. **37** (1991) 922.
10. *Murty, V. R. C., Bhat, J., Muniswaran, P. K. A.,* Biotechnol. Bioprocess Eng. **7** (2002) 367.
11. *Kwon, D. Y., Rhee, J. S.,* J. Am. Oil Chem. Soc. **63** (1986) 89.
12. *Markwell, M. A. K., Haas, S. M., Bieber, L. L., Tolbert, N. E.,* Anal. Biochem. **87** (1978) 206.
13. *Murty, V. R. C., Bhat, J., Muniswaran, P. K. A.,* Biotechnol. Lett. **26** (2004) 563.