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External Mass Transfer Model for a Recirculated Packed-Bed Batch Reactor on the Hydrolysis of Palm Olein Using Immobilized Lipase

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Abstract

The application of immobilized enzyme in organic synthesis is gaining popularity as it offers advantages over conventional chemical reactions. A recirculated packed-bed batch reactor (RPBBR) can be used for immobilized enzyme systems. However, external mass transfer limitation is significant in an RPBBR, especially at large scales. This study investigated the external mass transfer coefficients in an RPBBR. The effect of mass flow rate, one of the key parameters affecting the external mass transfer resistance, was considered. The hydrolysis of palm olein using immobilized lipase was used as a case study. A mass transfer correlation model of the form $J_D = K\,Re^{n-1}$ was developed based on the literatures. The Colburn factor, J_D, which is a function of Reynolds and Schmidt numbers, can be related to the external mass transfer coefficient, k_m. The values of K and n were determined by conducting experimental work in the RPBBR at different mass flow rates. It was found that the values of K and n are 0.056 and 1, respectively. Since the average mass transfer coefficients can be correlated in terms of dimensionless groups which characterize the flow conditions, this model can be used for the design of reactors, particularly scaling-up.

Keywords: external mass transfer coefficient - recirculated packed-bed batch reactor - immobilized lipase – hydrolysis - palm olein

1.0 Introduction

In recent years, immobilized enzymes have become a choice of interest in organic synthesis. Immobilized enzymes offer many advantages over free enzymes as they can be recovered and reutilized easily. Difficult separation processes can be avoided in the downstream processing of product purification. Besides that, immobilization can sometimes provide a better microenvironment for the activity of enzyme.

Various reactor configurations can be used for immobilized enzyme systems. Nowadays, recirculated packed-bed batch reactor (RPBBR) with single column or multicolumn packed-bed reactor is used commercially [1]. An RPBBR is shown in Figure 1. It consists of a fixed bed reactor with recycling system.

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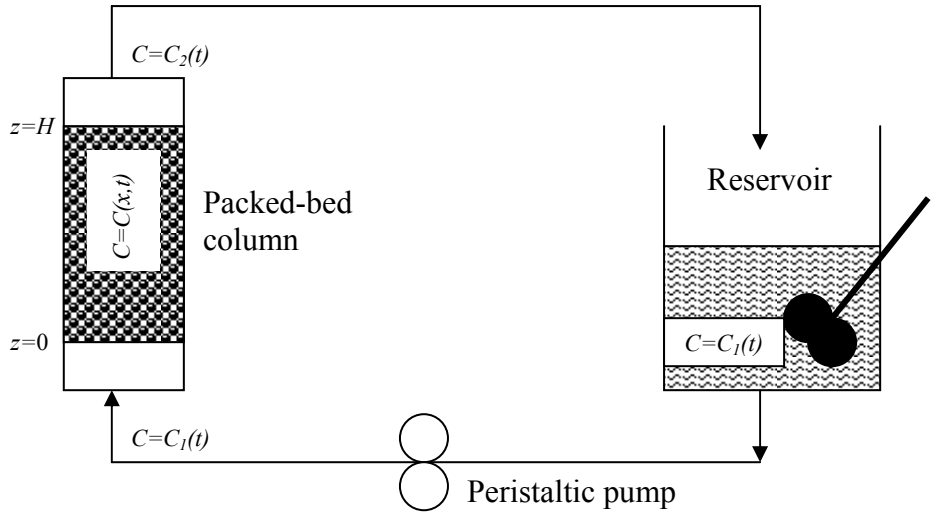

Figure 1 Schematic representation of an RPBBR [2].

An RPBBR enables enzyme reusability. The reactor construction is simple and it requires minimum start-up operation. Since the enzymes are enclosed in the reactor, enzymes recovery between batches is avoided. Therefore, risk of contamination and handling losses can be minimized. A faster reaction rate can also be achieved due to the high immobilized-lipase concentration in the reactor.

Most of the time, industrial practice involves large-scale reactor where mass transfer limitations are very significant. Therefore, a correlation model is important to estimate the mass transfer coefficient at different scales and operating parameters, to predict reactor performance and to aid scaling up besides overcoming other engineering constraints.

In order to develop an external mass transfer correlation model for an RPBBR, the hydrolysis of palm olein using immobilized lipase was used as a case study here. The hydrolysis reaction was conducted in an organic-aqueous phase. An organic-aqueous system was selected over a free-solvent system to reduce the viscosity of palm olein. Furthermore, the addition of solvent enables the concentration of substrates to be determined instead of taking the volumetric ratio of the substrates.

N-hexane was selected as the organic solvent due to its ability to dissolve most oils, fatty acids and derivatives of fatty acids. No surfactant or emulsifier was used in this study to ease downstream separation.

1.1 Development of a Mass Transfer Model

In a reactor packed with enzymes immobilized in a porous matrix, two transport processes occur. The first process is the transfer of the substrate from the bulk liquid phase to the surface of immobilized biocatalyst. The second process is the simultaneous diffusion and reaction of the substrate within the biocatalyst particles.

According to the film-theory, there is a presence of a fictitious laminar film next to the boundary of any surface in contact with a flowing fluid [3]. Therefore, when fluid flows

through a bed of particles, a near stagnant film of fluid is present on the surface of the particles where the fluid velocity is very low. In such regions around the exterior of particles, the substrate needs to be transported. This transport takes place primarily by molecular diffusion and it is called external mass transfer.

Murugesan and Sheeja [4] reported that in a co-current packed-bed reactor with up-flow mode of operation, the formation of surface film on the immobilized beads relatively reduces the observed rate of the reaction. Cooney [5], Nath and Chand [6] also found evidences of external film effects leading to convective mass transfer and therefore influences the reaction rates in immobilized packed-bed reactors.

Aksu and Bülbül [7]'s investigation of external mass transfer on phenol removal by immobilized cell shows that the external mass transfer rate and observed biodegradation reaction rate are affected by flow rate. In their experimental results, the pseudo first-order biodegradation rate constants increase while the phenol uptakes decrease with increasing flow rates. This is due to inefficient residence times between living cells and phenol at high flow rates, where the space time in the column is too short and the solute does not have enough time to diffuse into the pores of the particles. Other factors affecting the external mass transfer rate include substrate concentration, biomass quantity, and particle size.

In this study, the mass transfer model was developed based on the model developed by Aksu and Bülbül [7]. A few assumptions have been made during the development of this model as follows:

- The reaction follows a first-order rate (this is especially true at low substrate concentrations)
- The immobilized enzyme particles are spherical
- The packed-bed column has a plug flow with no axial dispersion
- The enzyme activity throughout the particle is uniform

1.2 Apparent Reaction Rate

A material balance for palm olein (substrate) in the packed-bed column was first developed as shown in equation (1).

$$\left(\frac{HQ}{W}\right)\frac{dC}{dz} \text{ x } 6 \text{ x } 10^{-2} = -r \qquad (1)$$

where r is the reaction (substrate consumption) rate (mg g^{-1} h^{-1}), Q the volumetric flow rate (ml min^{-1}), H the height of the column (cm), W the total amount of immobilized enzyme particles (g), and dC/dz the concentration gradient along the column length (mg l^{-1} cm^{-1}).

Since a first-order reaction rate was assumed, the relation between the apparent reaction rate and bulk substrate (palm olein) concentration in the column is given as:

$$r = k_p C \qquad (2)$$

where k_p is the apparent first-order reaction rate constant (l g^{-1} h^{-1}) or the observed reaction rate constant and C is the bulk substrate concentration (mg l^{-1}). Combining equation (1) and (2) gives:

$$(\frac{HQ}{W})\frac{dC}{dz} \text{ x } 6 \text{ x } 10^{-2} = - k_p C \quad (3)$$

Equation (4) is found by integrating equation (3) using boundary conditions at $z = 0$ of $C = C_{in}$, and at $z = H$ of $C = C_{out}$.

$$\ln (\frac{C_{in}}{C_{out}}) = \frac{W}{Q} k_p \text{ x } (10^3/60) \quad (4)$$

where C_{in} is the column inlet substrate (palm olein) concentration (mg l^{-1}) and C_{out} is the column outlet substrate (palm olein) concentration (mg l^{-1}). The concentration at the outlet of the packed-bed is therefore given by:

$$C_{out} = C_{in} e^{-N} \quad (5)$$

with N defined as

$$N = \frac{W}{Q} k_p \text{ x } (10^3/60) \quad (6)$$

Equation (5) only gives the relation between the inlet and outlet concentration of palm olein in the packed-bed column every time the fluid flows through the column. Since a recycling system is involved, the inlet concentration to the column changes for every cycle. Therefore, an overall mass balance for an RPBBR as developed by Mutlu and Gökmen [2] is as follows. Referring to Figure 1.1, if the reservoir is a perfectly mixed tank, the total mass balance gives

$$\frac{dV_{res}}{dt} = 0 \quad (7)$$

where V_{res} is the volume of the reacting solution in the reservoir (ml). The component balance in the reservoir gives

$$\frac{dC_1}{dt} = -\frac{1}{\tau}(C_2 - C_1) \quad (8)$$

where τ is the residence time (min) in the reservoir (V_{res}/Q), C_1 the concentration of palm olein (mg l^{-1}) in the reservoir, and C_2 the concentration (mg l^{-1}) at the outlet of the packed-bed column to be circulated back to the reservoir. Based on equation (5), C_2 can be defined as follows:

$$C_2 = C_1 e^{-N} \quad (9)$$

Substituting equation (9) into equation (8) gives

$$\frac{dC_1}{dt} = -\frac{1}{\tau}(C_1 e^{-N} - C_1) \quad (10)$$

Integrating equation (10) using boundary conditions of $V_{res} = V_{res}$ and $C_1 = C_0$ when $t = 0$ gives the change of palm olein concentration in the reservoir with time as

$$C_1 = C_0 \exp[-(e^{-N} - 1)t/\tau] \quad (11)$$

Based on equation (11), a plot of ln (C_1/C_0) versus time will give a slope term as follows:

$$\text{Slope} = - \frac{e^{-N} - 1}{\tau} \quad (12)$$

If a constant quantity of immobilized enzyme particles is used, the apparent reaction rate constant, k_p for each flow rate can be found from equation (6) when the value of N is known (from the slope as shown in equation (12)). k_p is the apparent rate constant which takes into account both the reaction and mass transfer phenomena.

1.3 Apparent Reaction Rate as a Function of Reaction and Mass Transfer Limitation

The mass transfer rate of the palm olein from the bulk liquid to the surface of the immobilized beads is proportional to the external mass transfer coefficient, area of mass transfer and the concentration difference between the bulk and the external surface of immobilized bead:

$$r_m = k_m a_m (C - C_s) \quad (13)$$

where r_m is the external mass transfer rate (mg g^{-1} h^{-1}), k_m is the external mass transfer coefficient (cm h^{-1}), and a_m is the surface area per unit weight of immobilized enzyme for mass transfer (cm^2 mg^{-1}), while C and C_s is the substrate concentration in the bulk liquid and at the surface of the immobilized particle (mg l^{-1}) respectively. The value of a_m can be determined using the following equation

$$a_m = 6/\rho_p d_p \quad (14)$$

with d_p as the particle diameter (cm) and ρ_p the particle density (mg cm^{-3}).

The first-order reaction rate at the surface of the enzyme particle can be written as follows:

$$r = kC_s \quad (15)$$

where k is the surface first-order reaction rate constant (l g^{-1} h^{-1}).

Since the rates of external mass transfer and reaction steps will be the same at steady state, equations (13) and (15) are equated and rearranged to give

$$C_s = \frac{k_m a_m C}{k + k_m a_m} \quad (16)$$

Substituting equation (16) into equation (15) and equating with equation (2), the effects of reaction and mass transfer on the apparent reaction rate constant, k_p is shown in equation (17).

$$k_p = \frac{kk_m a_m}{k + k_m a_m} \tag{17}$$

or

$$\frac{1}{k_p} = \frac{1}{k} + \frac{1}{k_m a_m} \tag{18}$$

The terms $(1/k)$ and $(1/k_m a_m)$ show the contributions of reaction and external mass transfer resistance on the k_p, respectively, at constant temperature.

1.4 Mass Transfer Correlation Model

The value of k is constant as far as this particular reaction is concerned and is independent of the operating parameter, particularly the mass flow rate and the scale of the system. However, the external mass transfer coefficient, k_m changes with parameters such as flow rate, reactor diameter and fluid properties. This in turn changes the apparent reaction rate. Therefore, a correlation is needed so that the mass transfer coefficient can be estimated at different operating parameters and during scale-up.

Average mass transfer coefficients between the bulk fluid and particle surface in the packed-bed reactor can be correlated in terms of dimensionless groups which characterize the flow conditions [6, 8, 9, 10]. The correlation of the external mass transfer coefficient, k_m, with variables such as flow rate, reactor diameter and fluid properties can be obtained by defining a dimensionless group as follows:

$$J_D = \frac{k_m \rho}{G}\left(\frac{\mu}{\rho D_f}\right)^{2/3} \tag{19}$$

where J_D is the Colburn factor, defined in terms of Schmidt number and Reynolds number.

The Schmidt number is the term in parentheses in equation (19) as follows:

$$N_{Sc} = \frac{\mu}{\rho D_f} \tag{20}$$

The symbols μ, ρ and D_f are the fluid viscosity (g cm^{-1} min^{-1}), density (g ml^{-1}) and diffusivity (cm min^{-1}), respectively.

The Reynolds number can be defined as

$$Re = \frac{d_p G}{\mu} \tag{21}$$

where d_p is the particle diameter (cm).

G is the mass flux (g cm^{-2} min^{-1}) and it can be calculated using equation (22) as follows:

$$G = \frac{Q}{a_c \varepsilon} \tag{22}$$

where Q is the volumetric flow rate (ml min^{-1}), a_c the cross-sectional area of column (cm^2) and ε the void fraction in a packed-bed.

A few correlations for mass flow rates are available, varying in the dependence of the Colburn factor, J_D, on Re, as follows:

$$J_D = K\, Re^{(n-1)} \tag{23}$$

Different mass transfer conditions have different values of K and n. The value of n varies from 0.1 to 1.0. Equating equation (19) and (23) and solving for the mass transfer coefficient gives

$$k_m = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1} G^n \tag{24}$$

or

$$k_m = A\, G^n \tag{25}$$

where $A = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-2/3}\left(\frac{d_p}{\mu}\right)^{n-1}$

Substituting equation (25) into equation (18) and rearranging it leads to the following equation:

$$\left(\frac{1}{k_p}\right) = \left(\frac{1}{A a_m}\right)\left(\frac{1}{G^n}\right) + \left(\frac{1}{k}\right) \tag{26}$$

Equation (26) can be analyzed for different values of n ranging from 0.1 to 1.0. A straight line of slope $1/(Aa_m)$ and intercept $1/k$ should be obtained if the experimentally measured values of $1/k_p$ versus $1/G^n$ for each value of n is plotted. The calculated values of A and k (the surface first-order reaction rate constant) from the graph are then used to get the values of k_m (using equation (25)) and an estimated k_p (using equation (18)). A trial-and-error procedure is repeated for all n values until the estimated value of k_p matches well with the experimental k_p.

2.0 Materials and Methods

The commercial immobilized lipase, Lipozyme TL IM (bead size 0.3-1.0 mm, wet bulk density 415 kg/m^3), was obtained from Novozymes. A commercial cooking oil (Seri Murni)

was used as the source of palm olein. Oleic acid, palmitic acid and linoleic acid (GC standard) were purchased from Sigma-Aldrich.

The batch stirred-tank reactor consisted of a water-jacketed vessel with a maximum capacity of 50 ml and a magnetic stirrer. A water bath (Grant Instruments, Cambridge, England) was used to maintain the temperature of the reaction mixture in the vessel. A peristaltic pump (Masterflex, Cole-Parmer) and a thermostat XK 16/20 (16 mm ID x 20 cm length) jacketed column from Pharmacia Biotech, Sweden was connected to the batch reactor to form a recirculated packed-bed batch reactor (RPBBR).

The reaction mixture (15 mL of palm olein, 23 mL of *n*-hexane, 2 mL of water) was first prepared and incubated at 55 ^{0}C and 200 rpm. 2 g of immobilized lipase was then packed into the jacketed column. A time zero-sample was taken. Reaction was initiated by switching on the peristaltic pump. Samples were taken at different time intervals and analysed for fatty acids. Experiments were repeated at three different flow rates (0.5, 5, 20 ml min^{-1}).

All the samples were analysed using gas chromatography. A Shimadzu GC-17A Version 3 (Kyoto, Japan) equipped with a flame-ionization detector (FID) was used. A Nukol fused-silica capillary column (15 m length x 0.53 mm ID x 0.5 μm film thickness, Supelco, USA) was used with nitrogen as the carrier gas. The injector and detector were set at 220 ^{0}C. The column temperature was programmed to rise from 180 ^{0}C to 215 ^{0}C at 12 ^{0}C/min and maintain for 4 minutes before rising again at 12 ^{0}C/min until it reaches 220 ^{0}C and stay for 2 minutes. The gas chromatography column was connected to Shimadzu CLASS-VP Chromatography Data System software (Columbia, USA). Calibration curves for the fatty acids were first prepared using external GC standard.

3.0 Results and Discussion

The effect of mass flow rate on the apparent rate of reaction was investigated in this study. Table 1 shows the experimental values of k_p at different flow rates, Q. According to Table 1, the apparent first-order reaction rate constants increase with the increasing flow rates in the range studied. As the flow rates increase, the turbulence of the flow increases and consequently reduces the mass transfer resistance.

Table 1 Apparent first-order reaction rate constants at various flow rates.

Q (mL/min)	k_p x 10^3 (l g^{-1} h^{-1})
0.5	6.123
5	21.927
20	26.998

Reynolds numbers, Schmidt numbers and mass fluxes were calculated from equations 20, 21 and 22 using μ = 0.207 g cm^{-1}min^{-1}, ρ = 0.74 g mL^{-1}, D_f = 5.59 x 10^{-3} cm min^{-1}, d_p = 0.065 cm, a_c = 2.01 cm^2 and ε = 0.04. Plot of $1/k_p$ against $1/G^n$ for n = 0.4, n = 0.6, n = 0.8 and n = 1.0 is illustrated in Figure 2.

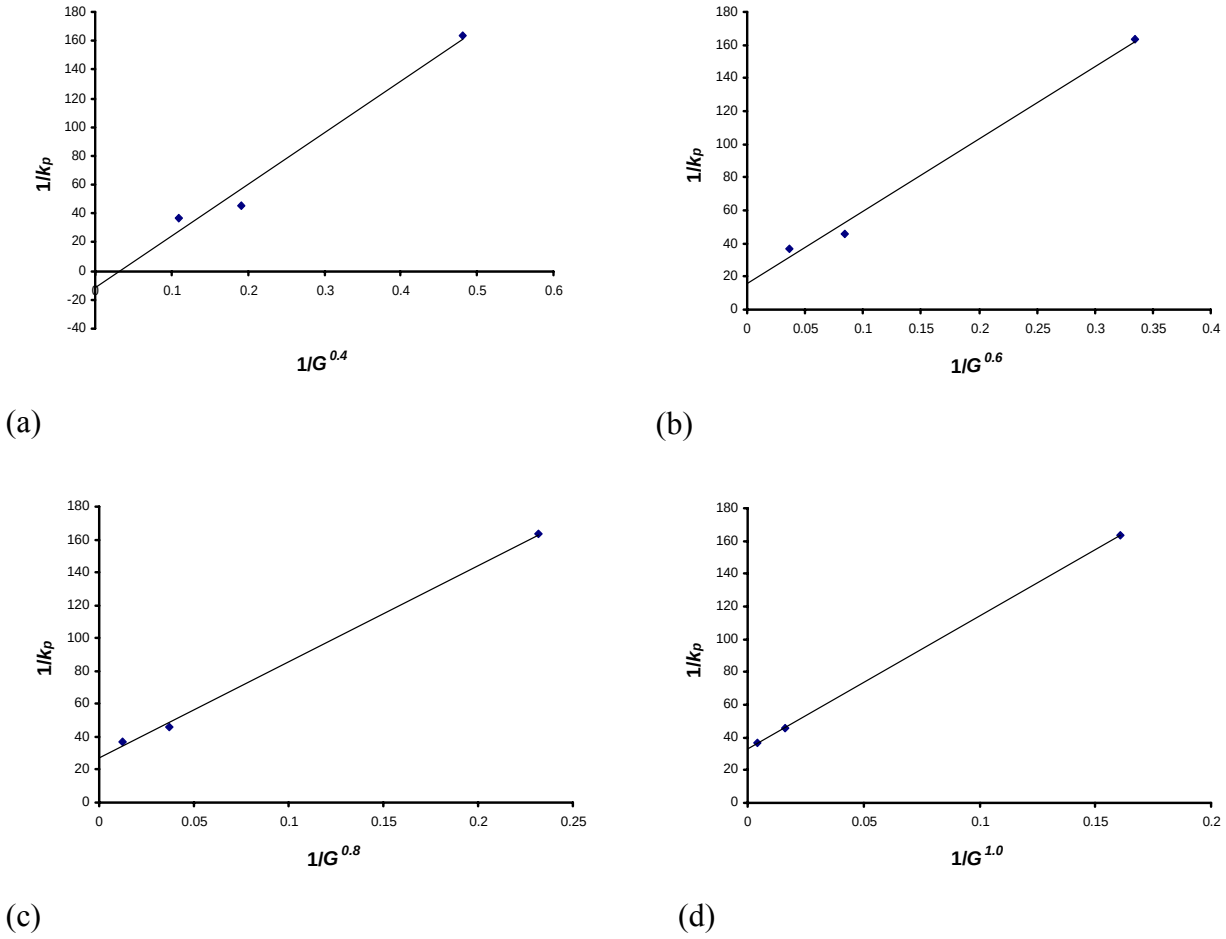

Figure 2 Plot of $1/k_p$ against $1/G^n$ for (a) $n = 0.4$; (b) $n = 0.6$; (c) $n = 0.8$; (d) $n = 1.0$.

The values of k and A at different n can be obtained from the plots in Figure 2. Since the intercept in Figure 2(a) has a negative value, it was not being further analyzed. Table 2 listed the values of k and A for $n = 0.6$, $n = 0.8$ and $n = 1.0$. These values were used to calculate for k_m (using equation (25)) and k_p (obtained from equation (18)), which were then compared with the experimental values of k_p. The results are tabulated in Table 3.

Based on the comparison in Table 3, it is found that the calculated k_p constants when $n = 1.0$ are quite closed to the k_p constants found experimentally. The value of K when $n = 1.0$ is 0.056. The results of this study show that a correlation $J_D = 0.056$ can be used to estimate the external mass transfer coefficient during the hydrolysis of palm olein in a reactor packed with immobilized lipase. The external mass transfer coefficient changes proportionally with the mass flux when the Schmidt number is constant and it is independent on the Reynolds number. As can be seen from Table 3, the external mass transfer coefficient increases when the flow rate increases. This is because higher flow rate gives higher turbulence and thus reduces the mass transfer resistance.

Table 2 Values of k and A calculated from the plots of $1/k_p$ against $1/G^n$ at various n values.

N	$k \times 10^3$ (l g^{-1} h^{-1})	$A \times 10^3$
0.6	65.032	10.226
0.8	36.782	7.676
1.0	30.104	5.561

Table 3 The comparison between experimental and calculated values of k_p at different n values.

Q (mL/min)	Experimental k_p x 10^3 (l g^{-1} h^{-1})	n = 0.6		n = 0.8		n = 1.0	
		k_m (cm h^{-1})	k_p x 10^3 (l g^{-1} h^{-1})	k_m (cm h^{-1})	k_p x 10^3 (l g^{-1} h^{-1})	k_m (cm h^{-1})	k_p x 10^3 (l g^{-1} h^{-1})
0.5	6.123	0.03	6.163	0.03	6.136	0.03	6.125
5	21.927	0.12	19.132	0.21	20.531	0.35	21.634
20	26.998	0.28	31.811	0.63	29.167	1.38	27.420

4.0 Conclusions

The external mass transfer limitation in a reactor packed with immobilized enzyme has significant effects on the overall reaction rate. This is especially true in large-scale reactors. A mass transfer correlation model in terms of dimensionless numbers is therefore very important in the design and simulation of reactor performance. Based on the results of this study, a correlation model J_D = 0.056 accurately predicted the experimental data for the hydrolysis of palm olein in an RPBBR using immobilized lipase. This model is valid for the range of flow rates used in this study. However, further studies should be carried out at a larger range of flow rates to ensure the applicability of this model.

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