Application of an Optimization Algorithm for Estimation of Substrate Mass Transfer Parameters for Immobilized Enzyme Reactions

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In this paper, a simple optimization methodology is applied for estimation of substrate mass transfer parameters including the overall external mass transfer coefficient, k_l, and the effective diffusivity, D_e, using experimental data for irreversible unireactant immobilized enzyme systems which follow Michaelis-Menten mechanism. The method utilizes a simple direct search optimization algorithm along with the numerical solution of the governing differential equation. The application of the method has been demonstrated through comparing the predicted values of k_l and D_e using the proposed method with experimental values reported in the literature for two different immobilized enzyme systems with irreversible reactions.

INTRODUCTION

Immobilization of enzymes on suitable support materials has resulted in their extended use in batch and continuous bioreactors. For immobilized enzymes, however, there are several factors which affect the observed kinetics that could be significantly different from the intrinsic kinetics of the free enzyme. These factors include interparticle and intraparticle diffusion limitations, steric and conformation effects, the partitioning of substrate between the support and bulk of the solution, conformation and spatial effects due to the immobilization mechanism which may cause disfiguration of the enzyme and micro-environmental effects due to the interactions of the support on the enzyme-substrate reaction resulting in a change in the enzymatic reaction mechanism. These effects depend on the properties of the support, the substrate and its concentration and on the immobilization procedure [1-3]. The mass transfer limitation effects on the observed reaction rates are due to external mass transfer resistance of substrate from the bulk fluid phase to the external surface of support pellets and internal mass transfer resistances due to pore diffusion [4,5]. The

extent of external mass transfer limitations depends on the diffusivity of the substrate in the bulk fluid phase, the velocity of the fluid phase over the support pellets, density and viscosity of the fluid phase and the substrate concentration [6,7]. The overall external mass transfer coefficient is represented by k_l. The extent of internal diffusion limitations depends on the shape and size of the support pellets, the external substrate concentration and the effective diffusivity, D_e, of the substrate within the support pellets. The effective diffusivity depends on the molecular diffusivity of the substrate in the support matrix and on the porosity, ε_p and tortuosity, τ, of the pellets.

For the purpose of bioreactor design, modeling and simulation and for process development, it is necessary to investigate the intrinsic kinetics for enzymatic reactions; i.e. reaction kinetics in the absence of mass transfer limitations [8]. Michaelis-Menten equation is the most common rate expression used for enzyme reactions [9]. This equation can also be used for immobilized enzymes. In the absence of mass transfer limitations, the kinetic constants in Michaelis-Menten equation, K_m and V_m, are intrinsic kinetic parameters, which due to the above mentioned factors, may not necessarily be the same as those for the free enzyme reactions [10-14].

There are different experimental methods to determine the intrinsic kinetic parameters for immobilized enzymes [8,15-19] which involve the measurement of initial reaction rates at different initial substrate concentrations under conditions where the external mass

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transfer resistance is eliminated by the high velocities of the fluid phase over the support pellets and the internal diffusional limitations are eliminated by reducing the size of the support pellets. Intrinsic kinetic parameters are, thus, obtained by Lineweaver-Burk plot. These methods require extensive experimental effort with pellets of different sizes.

A number of calculational methods have been proposed to determine the intrinsic kinetic parameters for immobilized enzymes with irreversible [2,20-23] and reversible reactions [8,24]. In one method [22], the system is so complex mathematically that creates difficulties when applied. In other methods [21], the maximum rate must be found by raising the bulk substrate concentration to levels sufficiently high that diffusion limitations are removed, however, as demonstrated in the article, this is not always possible. Another technique [2] can only be used if straight lines are obtained when experimental data are plotted by standard graphical methods. As indicated in this article [2], these plots often deviate from straight line behavior. Other methods [20,21] require a number of experimental points at low substrate concentrations (high extent of diffusional limitations) which may lead to experimental difficulties due to the low observed rates caused by low enzyme saturation. In another technique [23], the maximum rate can be determined only if the intrinsic Michaelis constant is known a priori. In another method [25], the reaction conditions are such that external mass transfer limitations are absent and initial reaction rates at low and high substrate concentrations and an effective diffusivity, D_e, are used along with reference plots for the effectiveness factors, Ef, and theoretical relationships utilizing Bischoff [26] and Aris [27] modulus to determine the intrinsic kinetic parameters. At low substrate concentration, the film diffusion can affect the observed activity, thus, leading to results with low accuracy.

Once the intrinsic kinetic parameters have been determined, it is necessary to specify the mass transfer parameters, k_l and D_e, to include the effect of external and internal mass transfer limitations in bioreactor design calculations. There are different methods to estimate the substrate mass transfer parameters. The following relationships have been proposed for estimation of D_e:

Petersen [28]: $\qquad D_e = \varepsilon_p^{3/2} D,$
Wakao and Smith [29]: $\quad D_e = \varepsilon_p^2 D,$
Satterfield [30]: $\qquad D_e = \frac{\varepsilon_p D}{\tau},$

where D is the molecular diffusivity of the substrate. Colburn correlations have also been used to estimate k_l in a liquid phase fixed bed reactor:

Wilson and Geankopolis [31]:

$$0.0016 < Re_p < 55 \quad k_l = \frac{1.09u}{\varepsilon_b} Pe_p^{-2/3}$$

$$55 < Re_p < 1500 \quad k_l = \frac{0.25u}{\varepsilon_b} Re_p^{-0.31} Sc^{-2/3},$$

McCune and Wilhelm [32]:

$$8 < Re_p < 20 \quad k_l = \frac{1.65u}{\varepsilon_b} Re_p^{0.507} Sc^{-2/3}$$

$$120 < Re_p < 1300 \quad k_l = \frac{0.687u}{\varepsilon_b} Re_p^{-0.327} Sc^{-2/3},$$

Rowe et al. [33]:

$$Re_p < 20 \quad k_l = \frac{D_s}{d}(2 + 0.76Sc^{1/3}Re_p^{0.33}),$$

where the dimensionless numbers are defined as:

$$Re_p = \frac{du\rho}{\mu}, \quad Sc = \frac{\mu}{\rho D_s}, \quad Pe_p = Re_p, \quad Sc = \frac{du}{D_s}.$$

u is the fluid apparent velocity, d is the diameter of the pellets, ρ is the fluid density, μ is the fluid viscosity, D_s is the substrate diffusivity in the fluid phase and ε_b is the bed porosity. The above correlations provide reasonable estimates of mass transfer parameters provided that the properties of the substrate, the fluid phase and the support pellets are known. Some of the pellets properties including porosity and tortuosity can only be obtained experimentally.

Experimental techniques are often employed to evaluate D_e. Effective diffusivity can be obtained by performing transient diffusion and equilibrium adsorption experiments using dilute solutions of the substrate and support pellets free of enzymes. From the measurement of the substrate concentration as a function of time, effective diffusivity of the substrate in the pellet can be obtained [7,34-36]. Transient diffusion experiments are often conducted with a high enough mixer speed so that the external mass transfer resistances can be neglected. Other experimental techniques for determination of D_e involve pulse and response analysis in tracer studies using support particles free of enzymes [37].

In this study, a simple direct search optimization algorithm utilizing random numbers is applied along with the numerical solution of the governing differential equations for the estimation of mass transfer parameters, D_e and k_l, for immobilized enzymes with irreversible reactions when intrinsic kinetic constants for the free enzyme reactions and experimental initial rate data for different initial substrate concentrations are available. The kinetic model for immobilized enzymes is described and the method for parameter estimation is illustrated with reference to experimental data from the literature.

KINETIC MODELING FOR IMMOBILIZED ENZYMES

The following assumptions are made in the development of the kinetic model for immobilized enzymes [38,39]:

1. Kinetics of the free enzyme is described by Michaelis-Menten equation:

$$v = \frac{V_m S}{K_m + S} , \qquad (1)$$

where v is reaction rate, V_m is maximum reaction rate, K_m is Michaelis constant and S is substrate concentration.

2. The enzyme is uniformly distributed over the support material.

3. The partition effect between the support and bulk fluid phase is neglected.

4. Temperature and effective diffusivity are constant within the support.

5. Steady-state conditions are developed.

6. Enzyme deactivation is neglected.

The following differential equation and associated boundary conditions express the dimensionless substrate concentration, C, in the pellet [10]:

$$\frac{d^2C}{dX^2} + \frac{g-1}{X}\frac{dC}{dX} = \phi^2 \frac{C}{1+\beta_b C}$$

at $X = 0$: $\dfrac{dC}{dX} = 0$

at $X = 1$:

$C = 1$ (without external mass transfer resistance)

$\dfrac{dC}{dX} = Bi(1-C)$ (with external mass transfer resistance)

$$\qquad (2)$$

where X is the dimensionless distance to the center or the surface of symmetry of the pellet and g is the pellet shape factor which for slab, cylindrical and spherical pellets is 1, 2 and 3, respectively. The dimensionless parameters are defined as follows:

$$C = \frac{S}{S_b}, \qquad X = \frac{x}{R}, \qquad \beta_b = \frac{S_b}{K_m},$$

$$\phi = R\sqrt{\frac{V_m}{K_m D_e}} \text{ (Theile Modulus)},$$

$$Bi = R\frac{k_l}{D_e} \text{ (Biot number)}.$$

In the above expressions, x is distance to the center, R is half-thickness of the pellet, S_b is substrate concentration in the bulk fluid phase, k_l is external mass transfer coefficient and D_e is effective diffusivity of the substrate in the pellet. Under steady-state conditions, the reaction rate can be evaluated from the concentration profile obtained from the solution of Equation 2 by either of the following methods:

1. From the rate of substrate diffusion at the pellet surface,

2. From the rate of mass transfer from bulk fluid phase to the external pellet surface,

3. From integration of reaction rate from the pellet surface to the center.

The corresponding equations for the above methods are given by Equations 3 to 5:

$$v = \frac{gD_e}{R}\frac{dS}{dx}\Big|_{x=R}, \qquad (3)$$

$$v = \frac{gk_l}{R}(S_b = S|_{x=R}), \qquad (4)$$

$$v = g\int_0^R \left[\frac{V_m S}{K_m + S}\right] x^{g-1}dx. \qquad (5)$$

To describe the mass transfer limitation effect on the overall reaction rate, the following definition for the overall effectiveness factor, Ef, is used [40]:

$$Ef = \frac{\text{reaction rate}}{\substack{\text{reaction rate in the absence of} \\ \text{internal and external resistances}}}, \qquad (6)$$

where the numerator is one of the relationships given by Equations 3 to 5 and the denominator is the reaction rate per unit volume of the pellet if the substrate concentration is taken as that in the bulk fluid phase. By employing Equation 5, the following expression for Ef is obtained [10]:

$$Ef = g(1+\beta_b)\int_0^1 \frac{C}{1+\beta_b C}X^{g-1}dX. \qquad (7)$$

NUMERICAL SOLUTION AND VERIFICATION OF THE KINETIC MODEL

Equation 2 was solved numerically using a finite difference formulation for a boundary value problem. The resulting non-linear system of algebraic equations was solved using Gauss-Seidel iterative algorithm with relaxation to obtain the dimensionless concentrations inside the pellet. The grid size for dimensionless distance to the center of the pellet was taken as 0.05. The dimensionless concentration profile can be used to obtain the effectiveness factor by numerical integration of Equation 7. Predicted values of initial reaction rates, v_o, could be obtained for different initial substrate

concentrations, S_{bo}, from which Lineweaver-Burk or Hanes-Woolf plot could be constructed. In the case of irreversible reactions, for example, v_o is given by:

$$v_o = Ef \frac{V_m S_{bo}}{K_m + S_{bo}}. \tag{8}$$

Alternatively, the initial reaction rate for the corresponding initial substrate concentration can be obtained by numerical differentiation using Equation 3 and the concentration profile within the pellet as obtained by the solution of Equation 2.

To test the validity of the model assumptions, the model predictions are compared with available experimental data from the literature. Table 1 provides the summary of the immobilized enzyme systems which were investigated in this study. Figure 1 represents a comparison of the model predictions with experimental results for case 1. The solid line is Hanes-Woolf plot in the absence of mass transfer limitation ($Ef = 1$) which shows significant deviations from experimental data. When internal diffusional limitations are taken into consideration, the agreement between predicted and experimental initial rate is reasonable over the entire range of initial substrate concentration. Figure 2 represents a comparison of the model predictions with experimental results for case 2. The dashed line in this figure represents model predictions using a constant effective diffusivity of 5.3×10^{-11} m^2/s. Good agreement between predicted and experimental initial rates is only observed at low initial substrate concentrations. The dotted line represents the model predictions using concentration dependent diffusivities [25] (Table 2) which shows a good agreement with experimental data over the entire range of initial substrate concentration. The correlation for concentration dependent diffusivities in Table 2 was developed using the effective diffusivities

Table 1. Summarized description of the immobilized enzyme systems investigated.

Case #	1	2
Enzyme	Amiloglucocidase	Amiloglucocidase
Support	Honeycomb ceramic slab	Porous spherical glass beads
R (m)	1.6×10^{-4}	1.65×10^{-4}
Substrate	Soluble starch	Dextrin
Product	Glucose	Glucose
Reactor	Stirred batch reactor	Recycling differential batch reactor
D_e (m^2/s)	3.67×10^{-12}	5.30×10^{-11}
k_l (m/s)	Negligible	Negligible
Reference	[11]	[25]
K_m	0.251 (kg/m^3)	1.2521 (μmol/cm^3)
V_m	2.4×10^{-2} (kg/s/m^3 cat)	147.63 (μmol/min/cm^3 cat)
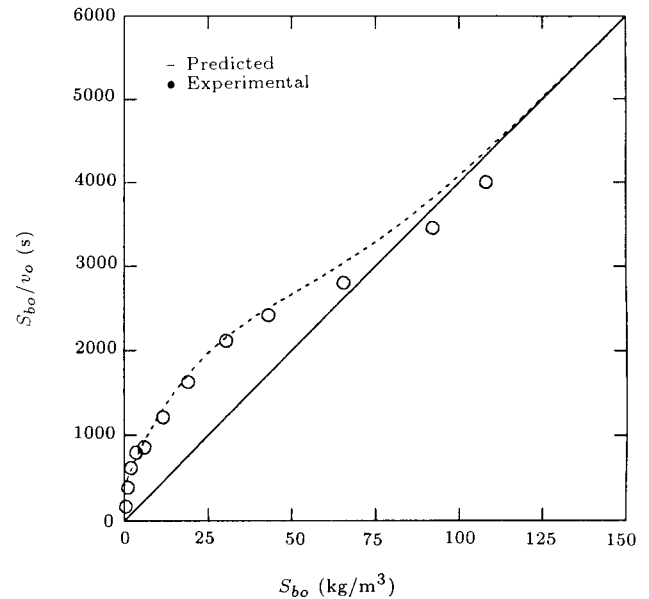
Figure 1. S_{bo}/v_o versus S_{bo} for case 1.
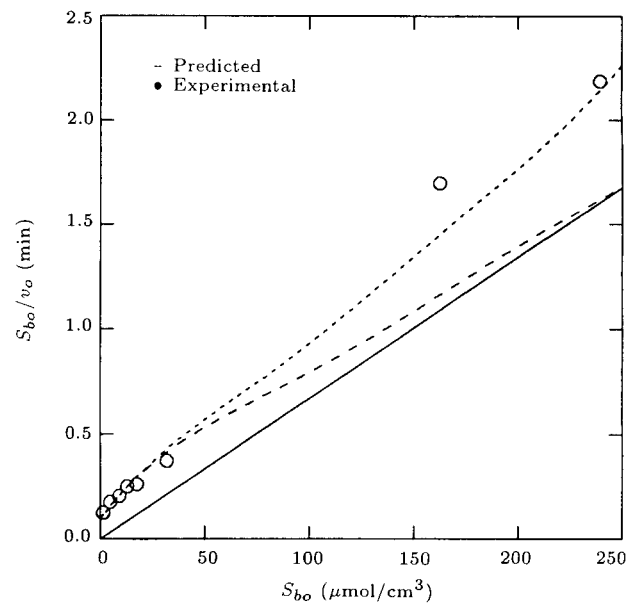
Figure 2. S_{bo}/v_o versus S_{bo} for case 2.

for different substrate concentrations as reported by Lee et al. [25].

OPTIMIZATION ALGORITHM FOR ESTIMATION OF INTRINSIC KINETIC PARAMETERS

The optimization method for parameter estimation is based on a direct search algorithm with systematic reduction of the search region [41]. The flowchart of the algorithm is presented in Figure 3. The input data for the program include the pellet size and shape, Michaelis-Menten constants, K_m and V_m,

Table 2. Variation of the experimental effective diffusivity with dextrin concentration for case 2, $D_e = 6.025 \times 10^{-11} \exp(-5.94 \times 10^{-3} S)$.

$S(\mu\text{mol}/\text{cm}^3)$	$D_e(\text{m}^2/\text{s})$
0	5.8×10^{-11}
43.48	4.7×10^{-11}
86.96	3.8×10^{-11}
173.91	2.3×10^{-11}
260.87	1.3×10^{-11}

and experimental initial reaction rates, v_o, at different initial substrate concentrations, S_{bo}. The parameters to be optimized are the effective diffusivity, D_e, and the external mass transfer coefficient, k_l. Initially, an upper and lower bound for each parameter is specified. The lower bound for non-negative rate constants is taken as a small positive number. The upper bound can be specified by an arbitrary high value that covers the range of values typically reported in the literature. The mid point for each parameter range is taken as the initial estimate of the optimum parameter values. Determination of the optimum parameters within the specified search region proceeds by selecting a parameter value from each range using random numbers. The governing differential equations are, then, solved numerically using the randomly selected parameters to obtain the initial reaction rates for a given set of initial substrate concentrations. The predicted initial rates are used to evaluate an objective function for optimization which is formulated as follows:

$$OF = \sum_{i=1}^{L}[v_{o,\text{experimental}} - v_{o,\text{predicted}}]^2, \qquad (9)$$

where $v_{o,\text{experimental}}$ and $v_{o,\text{predicted}}$ are the experimental and predicted initial rates, respectively, and L is the number of experimental measurements. The optimum parameter values are those which minimize the above objective function. The algorithm proceeds by another random selection of parameters from the search regions and subsequent evaluation of the objective function. The procedure is repeated $N1$ times for parameter values that are selected within the search region and the set of parameters which results in the least value for the objective function is stored as the optimum set for the search region. The proposed value for the number of times the parameters are randomly selected from the search region is between 100 and 500 [41] and in this study, 100 is used. The above procedure is repeated after a reduction of the search region; the range for each parameter is reduced by a factor of $(1 - \varepsilon)$. The proposed value for ε is between 0.005 and 0.05 [41] and in this study a value of 0.05 is used. The number of reductions of the search region, $N2$, used in this study
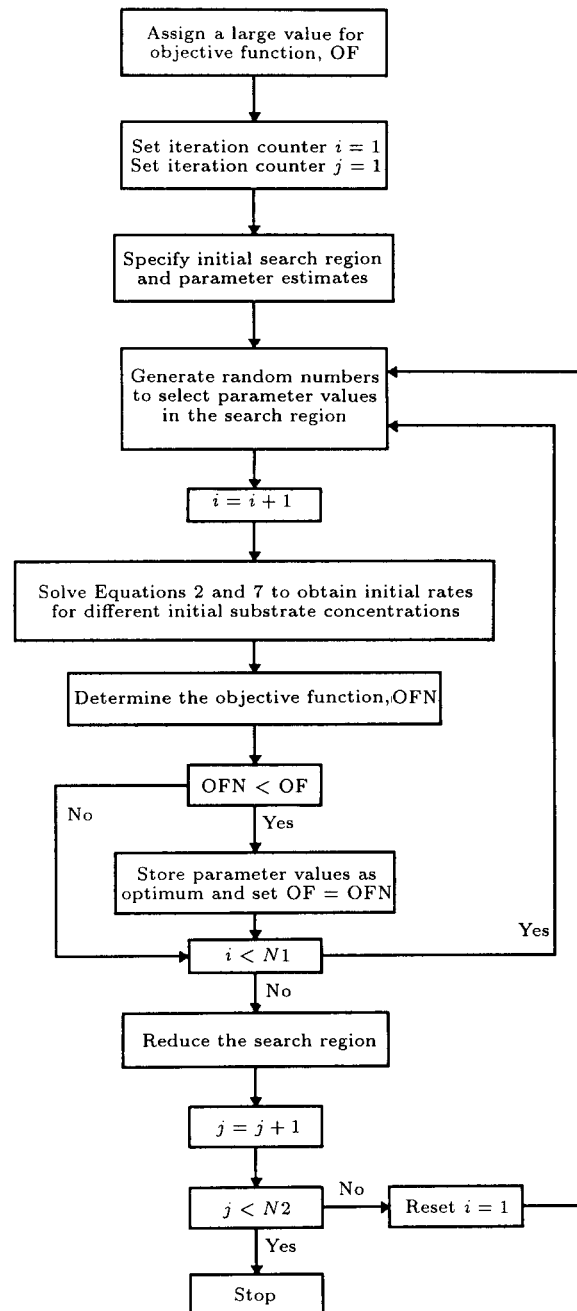
Figure 3. Flowchart for the optimization algorithm for parameter estimation.

was 200. The above optimization algorithm is quite simple to implement but requires a large computation effort; 20000 combinations of parameters are tested.

RESULTS AND DISCUSSION

Table 3 presents the experimental initial reaction rates at different initial substrate concentrations [11] for case 1. The mass transfer parameters, D_e and k_l, were obtained by the optimization algorithm using

Table 3. Experimental initial reaction rates at different initial substrate concentrations for case 1.

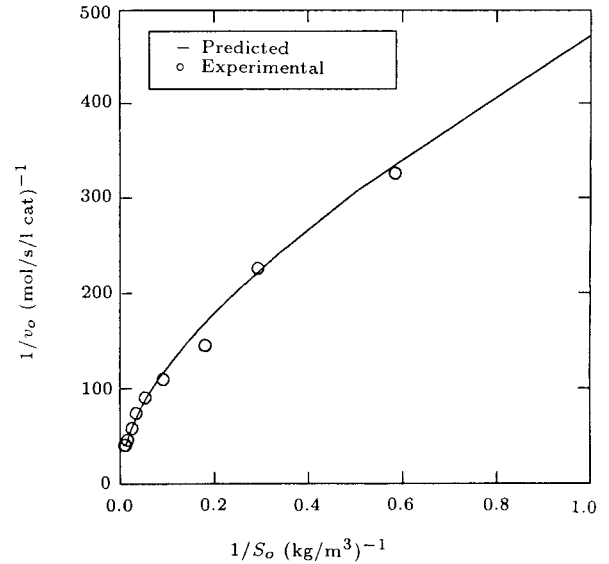| Experiment #	v_o (kg/s/m^3 Cat.)	S_o (kg/m^3)
1	1.942×10^{-3}	0.684
2	3.072×10^{-3}	1.709
3	4.414×10^{-3}	3.419
4	6.859×10^{-3}	5.555
5	9.102×10^{-3}	11.025
6	1.103×10^{-2}	18.803
7	1.349×10^{-2}	29.914
8	1.716×10^{-2}	41.453
9	2.184×10^{-2}	63.657

each of the experimental data points ($L = 1$ in the objective function) and are reported along with the optimum values of the objective function, $P_{\min}$, in Table 4. In all cases, the optimized values of D_e were in good agreement with the reported value of 3.67×10^{-12} m^2/s. Furthermore, the relatively large values for k_l (order of 10^{-1} m/s) indicate that external mass transfer limitations are negligible. When all the experimental points were considered in the formulation of the objective function ($L = 9$), the obtained value of D_e was also in very good agreement with the experimentally reported values. The initial reaction rates at different initial substrate concentrations for case 1 were predicted using the optimized mass transfer parameters (last row of Table 4) and are compared with experimental values in Figure 4 which show a good agreement between predicted and experimental values.

Table 5 presents the experimental initial reaction rates at different initial substrate concentrations [25] for case 2. The mass transfer parameters, D_e and k_l were obtained by the optimization algorithm using each of the experimental data points ($L = 1$ in the objective function) and are reported along with experimental effective diffusivities in Table 6. The experimental effective diffusivities for this case were dependent on the substrate concentration as reported in Table 2. In all cases, the optimized values of D_e

Table 4. Optimized mass transfer parameters for case 1 and the corresponding objective function values.

Experimental Data Used	D_e (m^2/s)	k_l (m/s)	$P_{\min}$
1	4.839×10^{-12}	8.314×10^{-2}	9.3×10^{-16}
2	3.011×10^{-12}	5.678×10^{-1}	2.7×10^{-16}
3	3.847×10^{-12}	7.525×10^{-1}	1.1×10^{-16}
4	5.258×10^{-12}	2.510×10^{-1}	2.0×10^{-18}
5	4.006×10^{-12}	2.057×10^{-1}	6.8×10^{-16}
6	3.545×10^{-12}	4.937×10^{-1}	7.8×10^{-16}
7	3.284×10^{-12}	3.344×10^{-1}	2.2×10^{-17}
8	3.794×10^{-12}	1.209×10^{-1}	5.0×10^{-16}
9	3.965×10^{-12}	9.395×10^{-1}	5.6×10^{-17}
1 to 9	3.825×10^{-12}	1.240×10^{-1}	4.6×10^{-6}

were in reasonable agreement with the experimental values. The optimized values for k_l were 4 order of magnitude smaller than those for case 1, however, they are sufficiently large enough (Biot numbers over 100) that external mass transfer limitations can be assumed to be negligible. When all the experimental points
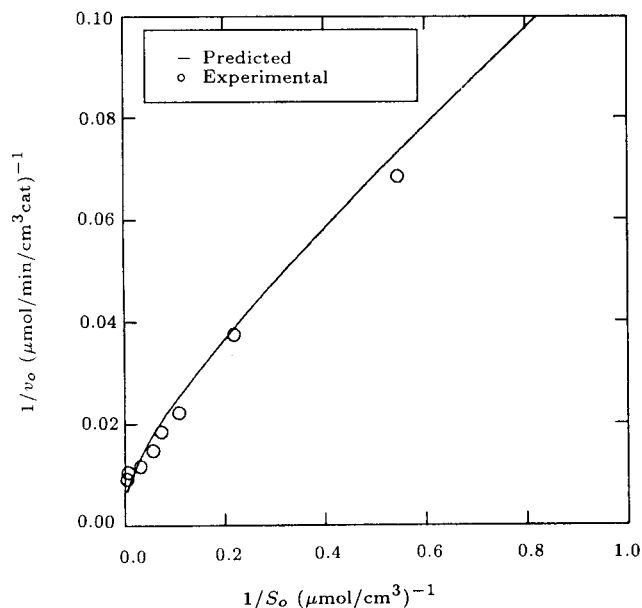

Figure 4. $1/v_o$ (mol/s/L Cat.)$^{-1}$ versus $1/S_o$ (kg/m^3)$^{-1}$ for case 1.

Table 5. Experimental initial reaction rates at different initial substrate concentrations for case 2.

Experiment #	$v_o(\mu\text{mol/min/cm}^3\text{Cat.})$	$S_o(\mu\text{mol/cm}^3)$
1	108.34	238.72
2	94.64	161.67
3	85.41	32.29
4	67.70	17.93
5	54.17	13.67
6	44.94	9.17
7	26.66	4.57
8	14.62	1.83

Table 6. Optimized mass transfer parameters and experimental effective diffusivity for case 2.

Experimental Data Used	$D_{e,opt}$ (m^2/s)	$D_{e,exp}$ (m^2/s)	$k_{l,opt}$ (m/s)
1	1.542×10^{-11}	1.530×10^{-11}	6.143×10^{-5}
2	1.537×10^{-11}	2.382×10^{-11}	9.480×10^{-5}
3	7.403×10^{-11}	5.005×10^{-11}	5.210×10^{-5}
4	9.702×10^{-11}	5.437×10^{-11}	4.389×10^{-5}
5	3.767×10^{-11}	5.570×10^{-11}	1.281×10^{-4}
6	4.713×10^{-11}	5.717×10^{-11}	7.663×10^{-5}
7	5.707×10^{-11}	5.868×10^{-11}	4.540×10^{-5}
8	8.375×10^{-11}	5.962×10^{-11}	6.552×10^{-5}
1 to 8	4.683×10^{-11}	–	2.395×10^{-5}

Figure 5. $1/v_o$ $(\mu\text{mol}/\text{min}/\text{cm}^3$ $\text{Cat.})^{-1}$ versus $1/S_o$ $(\mu\text{mol}/\text{cm}^3)^{-1}$ for case 2.

were considered in the formulation of the objective function ($L = 8$ and D_e independent of substrate concentration), the obtained value of D_e was in good agreement with the experimentally reported values in the low substrate concentration range. Alternatively, one could propose a model for D_e as a function of substrate concentration (similar to that proposed in Table 2) and proceed to obtain the optimized model parameters. The initial reaction rates at different initial substrate concentrations for case 2 were predicted using the optimized mass transfer parameters (last row of Table 6) and are compared with experimental values in Figure 5 which illustrate a good agreement between predicted and experimental values.

CONCLUSIONS

The methodology outlined in the paper provides a new approach for estimation of mass transfer parameters in immobilized enzyme systems. The knowledge of the intrinsic kinetic parameters is pre-requisite information for the optimization scheme. This information can be obtained experimentally under conditions where external and internal mass transfer limitations are absent. It should be emphasized, however, that the intrinsic kinetic parameters for immobilized enzymes might in many instances be different from those of the enzyme in its free state due to conformational, electrostatic, and steric effects caused by immobilization.

In this study, a simple optimization algorithm was applied for estimation of mass transfer parameters using initial rate data for irreversible unireactant immobilized enzymes provided that the intrinsic kinetic

parameters for the Michaelis-Menten rate expression are known a priori. The optimized parameters were in good agreement with experimentally reported values.

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