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Prediction of the fixed-bed reactor behaviour using dispersion and plug-flow models with different kinetics for immobilised enzyme

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Abstract

The behaviour of fixed-bed reactors, which have an immobilised enzyme on the packing surface, was studied considering steady-state conditions and external mass transfer resistance in the fluid around catalyst spherical particles. Solutions were obtained by integration of the plug-flow model equation and by the orthogonal collocation method of the second order differential equation of the axial dispersion flow model. Both models were analysed for lactose hydrolysis with β-galactosidase immobilised on chitosan using different kinetic reaction mechanisms after determining the specific parameters. The calculated results show the importance of the hydrodynamic and kinetic reaction parameters for error reduction in the prediction of the experimental behaviour.

Keywords: Fixed-bed reactor; Plug-flow model; Axial dispersion flow; Kinetic reaction parameters

1. Introduction

The enzyme immobilisation is a procedure for obtaining insoluble catalyst, which has the advantage that it can be used in continuous reactors. Then, this reaction unit is a critical point in the industrial process production, which require the design optimisation and the operation control. Substrate conversion is dependent of several reactor conditions and characteristics that can be associated in mathematical models. Moreover, a variety of factors that affect the reactor performance such as enzyme reactions, chemical and physical properties of substrates and products and flow characteristics can be defined as long as the right problem can be solved. Therefore, the use of model solutions showing the reactor behaviour can improve the knowledge of the process and the selection of operating conditions [1].

Packed-bed reactors are often used for reaction system with product inhibition. In this case the efficiency is larger since the inhibition effect decreases due to the low difference between substrate and product concentrations in the whole reactor.

For this reactor, mathematical models have taken into account mass transport equations according the operating conditions, such as the hydrodynamic conditions, the plug-flow or axial dispersion flow; the external or/and internal mass transfer resistance and the kinetic reaction rate. Solutions of the equation system have accurately determined experimental results and the effect of different variables and conditions.

In several previous cases, equations were often theoretically solved following different approaches in order to simplify the problem. As a result, some variables were disregarded or neglected to obtain a single equation that can be solved or adjusted fitting experimental data [2]. The kinetic constants and correlation of the mass transfer coefficient were modified; fitting different values with the plug-flow model in order to optimise the adjustment. On the other hand, due to the low substrate concentration, the model was used after the Michaelis–Menten equation, was simplified to first-order kinetic equation [3]. In other cases the mass transfer effect was not considered in the studies [4,5].

Numerical methods are applied to solve the partial differential equations of an axial dispersion model [6,7] or complex reaction kinetics [8]. Thus, important factors, such as difusional resistances, effects of backmixing or complex kinetic models, in some working ranges can be included in order to improve the theoretical values obtained.

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Nomenclature

a	surface area of particles per unit volume (cm^{-1})
A_{jk}, B_{jk}	coefficients of matrices A and B at the collocation point
C	constant of Chilton and Colburn equation
d_p	particle diameter (cm)
D_e	axial dispersion coefficient ($cm^2\ s^{-1}$)
$D_{L,S}$	substrate diffusion coefficient in water ($cm^2\ s^{-1}$)
E_0	initial enzyme concentration (g protein g^{-1} support)
g	exponent of Eq. (14)
G	glucose concentration
k'	intrinsic constant for first-order kinetic equation ($M\ s^{-1}\ g^{-1}$ protein)
k'_2	intrinsic constant for Michelis–Menten kinetic equation ($M\ s^{-1}\ g^{-1}$ protein)
k_L	mass transfer coefficient ($cm\ s^{-1}$)
K'_m	intrinsic Michaelis–Menten constant (M)
k'_P	intrinsic inhibition constant (M)
L	reactor length (cm)
n	number of internal collocation point
P	galactose concentration (M)
Pe	Peclet number, $u\ L/D_e$
q_i	coefficient of the polynomial equation
r_S	reaction rate term
r^2	coefficient of determination
Re	Reynolds number, $d_p\ U\ \delta_L/\mu_L$
S	substrate concentration in bulk solution (M)
Sc	Schmidt number, $\mu_L/D_{L,S}\ \delta_L$
S_e	fit standard error
S_i	substrate concentration in catalyst surface (M)
S_0	substrate concentration at reactor inlet (M)
St	modified Stanton number (dimensionless mass transfer coefficient), $k_L\ a\ L/u$
S_j	polynomial defined by Eq. (13)
T	temperature (K)
u	superficial fluid velocity ($cm\ s^{-1}$)
V'_{max}	intrinsic maximum reaction rate ($M\ s^{-1}$), $E_0\ k'_2$
V_{inj}	injection volume of glucose
z	axial coordinate for reactor (cm)
z_j	internal collocation point function
z^*	dimensionless axial coordinate, z/L

Greek symbols

α	dimensionless substrate concentration, S_i/S
δ_L	solution density ($g\ cm^{-3}$)
δ_p	particle density ($g\ cm^{-3}$)
ε	void fraction of packed-bed reactor
μ_L	solution viscosity ($g\ cm^{-1}\ s^{-1}$)
ω_{sop}	weight of support (g)

The efficiency of the reactor with β-galactosidase immobilised on chitosan in a tubular fixed-bed reactor for the hydrolysis of lactose was experimentally determined and calculated, modifying some transport parameters according to the operation conditions [9].

The objective of this study was to apply a methodology for determination of the best set of equations describing the process.

2. Theory

The mathematical model for the substrate concentration profile in one direction (z) of an isothermal tubular reactor of a length, L with axial dispersion and external mass transfer resistance in steady-state is the model often used [10]. The axial dispersion coefficient, D_e, the superficial velocity in the reactor, u, are also variables defined.

After using dimensionless variables, $z^* = z/L$ and S_i by $\alpha = S_i/S$, equations can be written in the following form:

$$\frac{1}{Pe}\frac{d^2S}{dz^{*2}} - \frac{dS}{dz^*} - St\,S(1-\alpha) = 0 \quad (1)$$

with boundary conditions:

$$z^* = 0^+, \qquad S = S_0 + \frac{1}{Pe}\frac{dS}{dz^*} \quad (2)$$

$$z^* = 1, \qquad \frac{dS}{dz^*} = 0 \quad (3)$$

where Pe is the Peclet number and St the modified Stanton number, which are defined in the nomenclature.

In the case of plug-flow model, where dS/dz^* is constant and $d^2S/dz^{*2} = 0$, Eqs. (1)–(3) become:

$$\frac{dS}{dz^*} + St\,S(1-\alpha) = 0 \quad (4)$$

with the condition:

$$z^* = 0^+, \qquad S = S_0 \quad (5)$$

The substrate (S) mass balance with the substrate in the interface S_i, the mass transfer coefficient, k_L and the specific area of the catalyst particle, a, are controlling the reaction rate r_S, by:

$$k_L a(S - S_i) = r_S \quad (6)$$

The equations to obtain the reaction rate considering different kinetics are shown in Table 1. Michaelis–Menten with inhibition (type III) equation is the most acceptable for working with product inhibition, which is the case of the proposed enzyme [11].

The substrate S changes in both models, namely the plug-flow and the dispersion model according to the rate of kinetic reactions. For this reason, the main equation of each model and Eq. (6) must be solved with different equations given in the Table 1 in order to consider the α variations

Table 1
Reaction rate equations

Type of reaction	r_S
(I) First-order	$k' S_i$
(II) Michaelis–Menten	$\frac{V'_{max} S_i}{K'_m + S_i}$
(III) Michaelis–Menten with competitive inhibition by product	$\frac{V'_{max} S_i}{K'_m(1 + (P/k'_p)) + S_i}$

indicated in the Appendix A [12]. The mathematical system is a first-order differential equation for plug-flow conditions which can be integrated with more or less difficulty according to the kinetic reaction considered. However, the model for a dispersion flow in the reactor involves a second-order partial differential equation, which can be solved with the method of orthogonal collocation with n internal points [13]. This method is based on expanding the variable S in terms of z_j^* using a series of known functions S_j to obtain an approximate solution in the domain $z^*(0,1)$. The series S_j is a polynomial where the first term satisfies boundary conditions of the problem and each of the additional terms satisfies the homogeneous boundary conditions when the RHS is equal to zero. In the collocation method the points are automatically picked by requiring that polynomials must be orthogonal to each other so that at a set of n internal points the series S_j are the exact solution with a weighted residual equal zero. Then the unknown coefficients q_i of the polynomials can be calculated for these collocation points and the solution can be obtained. The first and second derivatives of polynomials generate the coefficients A_{jk} and B_{jk} of matrices A and B at the collocation point. Eqs. (1)–(3) in this form, can be written:

$$\text{For } j = 2, \ldots, n+1 \quad \frac{1}{Pe}\sum_{k=1}^{n+2} B_{jk} S_k - \sum_{k=1}^{n+2} A_{jk} S_k - St\, S_j(1 - \alpha_j) = 0 \tag{10}$$

$$\text{For } j = 1 \quad z^* = 0^+, \quad S_1 = S_0 + \frac{1}{Pe}\sum_{k=1}^{n+2} A_{1k} S_k \tag{11}$$

$$\text{For } j = n+2 \quad z^* = 1, \quad \sum_{k=1}^{n+2} A_{(n+2)k} S_k = 0 \tag{12}$$

$$S_j = \sum_{i=1}^{n+2} q_i z_j^{*(i-1)} \tag{13}$$

Using $n = 6$ and weighting function $W = 1$, the collocation matrices A and B were generated [13]. The Eqs. (10)–(12), obtained after this procedure, represent a group of non-linear algebraic equations that were solved by Gauss method. Since these results are used to predict experimental concentrations obtained at the exit of the reactor, the concentration profile inside the reactor is not necessary to know and only the value in the outlet flow is important to calculate. Therefore, Eqs. (10)–(12) were solved to obtain the S value in the last point for different flow velocities.

2.1. Estimation of the mass transfer coefficient

The mass transfer coefficient was estimated by the Chilton and Colburn correlation [14] as function of the superficial velocity u, given by:

$$k_L = C \frac{D_{L,S}^{2/3}}{d_p^g} \left(\frac{\delta_L}{\mu_L}\right)^{(2/3-g)} u^{(1-g)} \tag{14}$$

where C is $1.09/\varepsilon$ and ε the average void fraction, $D_{L,S}$ the liquid-phase diffusivity of lactose, d_p the particle diameter, g is equal to 2/3 [4], and consequently k_L is independent of the density (δ_L) and viscosity (μ_L) of the lactose solution as a result of an exponent value equal to zero for these variables. These are particular values for Reynolds numbers (Re) between 1.6 E-3 and 55 and Schmidt numbers (Sc) between 165 and 70 600, which are ranges that correspond to numbers calculated at experimental system conditions. The surface area of particles per unit volume of packed-bed was estimated as $a = 6(1 - \varepsilon)/d_p$.

3. Materials and methods

Lactozym 3000 (*Kluyveromices fragilis* β-galactosidase) was obtained from Novo (Denmark). The kit for the glucose enzymatic determination was obtained from Wiener Lab (Argentina). Crab Shells chitosan and Sodium tripolyphosphate of practical grade were obtained from Sigma (USA). The other chemical reagents were analytical grade from Mallinckrodt or Merck (USA).

3.1. Enzyme immobilisation and kinetic parameters

The β-galactosidase was immobilised on chitosan beads using glutaraldehyde as it was described in a previous report [15]. Beads had an average diameter (d_p) of 0.22 cm and a density (δ_p) of 1.102 g cm^{-3}. The initial enzyme concentration (E_0) was equal to 0.021 g protein g^{-1} support. The kinetic parameters, fit standard errors (S_e) defined in Appendix A and coefficients of determination (r^2) were calculated by non-linear regression of experimental data with integrated equations of the reaction rate in a batch reactor [12]. Experimental values were obtained at 43 °C with negligible diffusion effects; the maximum velocity for a support weight, ω_{sop}, was $V'_{max} = k'_2 \omega_{sop} E_0$.

3.2. Packed-bed reactor

A (14.0 cm × 1.2 cm) column with a jacket of water recirculation and a heating water bath were used with

immobilised enzyme beads at 43 °C as the isothermal packed-bed reactor. More details can be found in the study presented previously [9]. Product (glucose) concentrations in the outlet flow were measured after feeding at constant flow rate in a range of 0.038–0.134 ml s^{-1}, by means of a peristaltic pump, solutions with different lactose concentrations, S_0 (M): 0.073–0.146–0.219–0.292, in 0.025 M KH_2PO_4 and 0.025 M Na_2HPO_4 buffer of pH 6.86. The weight of catalyst beads in the reactor was 10.66 g and the average void fraction ε of the packed-bed was equal to 0.39.

3.3. Estimation of axial dispersion coefficient

The axial dispersion coefficient (D_e) was estimated experimentally with the method used by Levenspiel [16], measuring the longitudinal spreading of the glucose concentration (G) in the output stream $R(t)$ during the period of time (t) that is detected. The solution of transient equation for the dispersed model with the condition ($G_{z=0} = G_0$) has a Gaussian distribution function. The relationship between the variance (the second moment with respect to the mean value) of the concentration versus the dimensionless time curve and the Peclet number (Pe) is $\sigma^2 = 2/Pe[1 - 1/Pe(1 - e^{-Pe})]$. The signal for the outlet pulse was obtained with a differential refractometer LDC Model 1107 Milton Roy Co. (Florida, USA) on line, it is showed in the Fig. 1 for different injection volume, V_{inj} of glucose (0.050, 0.075 and 0.100 ml) at a superficial velocity $u = 0.024$ cm s^{-1}. The area under the curve of response signal versus time was obtained by integration ($\Sigma G_i t_i / \Sigma t_i$) to determine σ^2, which was used to calculate Pe (corresponding a mean Pe equal to 8.7, with a estimate standard deviation equal to 0.1 for the velocity of Fig. 1) and then from this number the D_e [17]. Pe values calculated after examining the corresponding response curves that obtained for 10 different flow conditions and tracer quantities are given in Table 2. A $D_e = 0.038$ cm^2 s^{-1} were obtained from the average values.

Table 2
Experimental Pe numbers obtained in the axial dispersion coefficient determination

C_0 (g l^{-1})	V_{inj} (ml)	$\bar{t}$ (min)	σ^2	Pe[a]
5.0	0.100	9.62	20.43	9.06
5.0	0.075	10.89	29.49	8.04
5.0	0.050	10.69	24.56	9.31
10.0	0.100	10.08	23.05	8.82
5.0	0.100	9.63	20.27	9.15
5.0	0.050	10.67	24.58	9.27
5.0	0.075	10.63	26.95	8.39
10.0	0.100	10.60	26.57	8.45
10.0	0.100	10.60	26.57	8.45
10.0	0.100	10.60	26.57	8.45

[a] $Pe_{average} = 8.74$; S.D. = 0.14.

4. Results and discussion

Experimental values of the lactose conversion with immobilised β-galactosidase on chitosan beads as function of the superficial velocity in the reactor were obtained for different substrate concentration [9]. Theoretical values were predicted using the same experimental conditions in the two models with each kinetic equation. The kinetic constants, which were determined in a work before, are in Table 3 [12].

Experimental and predicted conversion values obtained for S_0 (M): 0.073 and 0.292 are given in Table 4, with the corresponding S_e in the case of the more complex equations [18], which is the axial dispersion model and the type III kinetics.

S_e for other cases, for different initial substrate concentration and an overall standard error for all concentrations

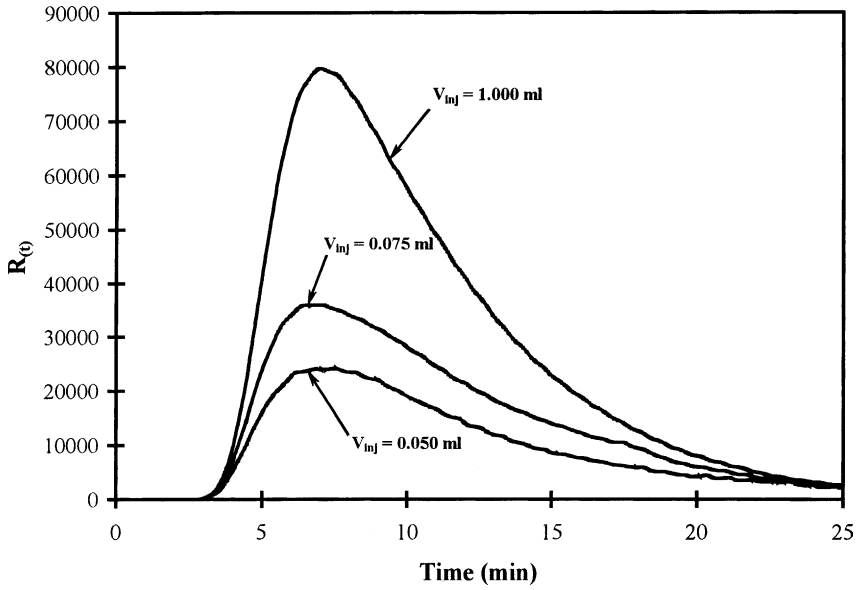

Fig. 1. Experimental curve of response signal vs. time obtained for different injection volumes of tracer.

Table 3
Kinetics parameters

Type of reaction	r_S
(I) First-order	$k' = 6.01$ E-3 M (glucose) $s^{-1}\,g^{-1}$ protein $r^2 = 0.556$ $S_e = 0.501$
(II) Michaelis–Menten	$k'_2 = 1.01$ E-2 M (glucose) $s^{-1}\,g^{-1}$ protein $K'_m = 0.141$ M $r^2 = 0.735$ $S_e = 0.136$
(III) Michaelis–Menten with competitive inhibition by product	$k'_2 = 1.30$ E-2 M (glucose) $s^{-1}\,g^{-1}$ protein $K'_m = 0.137$ M $k'_p = 0.234$ M $r^2 = 0.916$ $S_e = 0.085$

Table 5
Fit standard error between experimental data and predicted values for each kinetic reaction with the two models

Model	S_0 (M)	Kinetic type		
		I	II	III
Plug-flow	0.073	0.067	0.021	0.036
	0.146	0.024	0.086	0.014
	0.219	0.076	0.144	0.031
	0.292	0.137	0.204	0.051
	Overall	0.085	0.132	0.035
Axial dispersion	0.073	0.086	0.016	0.019
	0.146	0.037	0.037	0.026
	0.219	0.063	0.072	0.022
	0.292	0.115	0.102	0.023
	Overall	0.079	0.065	0.024

based on a dimensional conversion values (between 0 and 1) are given in Table 5. It is observed that the type III kinetics is the principal factor that reduces the S_e values, and improvement in the model fit is greater when using this kinetics than when using the axial flow model.

The combination of the axial dispersion model and the kinetic equation of Michaelis–Menten with a competitive product inhibition (type III) resented the smallest standard error. Besides, grouping these equations in the solution system, the S_e errors of the results obtained were similar for the different initial concentrations tested.

Figs. 2 and 3 show the conversion of the experimental data and calculated values for different kinetics in both models at different flow rates. Since reaction rate is directly proportional to the concentration changes, the kinetic equation type I show the more detectable variation of substrate conversion, which is not applied in this case. Therefore, the condition $K'_m \gg S$ at the initial concentration that simplifies the Michaelis–Menten equation in a kinetic equation of first-order could not be used [8]. The conversion values calculated with the kinetics equation, type II, are higher than experimental values since the product inhibition that reduces the substrate access at active site of enzyme was not considered in the model. The difference is larger for the values obtained with plug-flow model than those obtained with axial dispersion model. The latter system considered the lower substrate concentration for the reaction term because of the mixing flow in the reactor [19]. Finally, the best value was estimated using a kinetic equation type III [8]. This system also shows in Fig. 3 that the behaviour was similar not only with different substrate concentration but also with the velocity in all the ranges used. This also is indicated with the residual values shown in Table 4.

Using the combination of axial dispersion model and type III kinetics, the experimental values with smaller errors could be estimated for different initial concentrations. Similar errors were presented with the same kinetics equation in the plug-flow model when the C values in the Eq. (14), were previously adjusted with experimental values at each concentration at low superficial velocity and small substrate conversion [9]. The correlation of mass transfer coefficient was modified to reduce the effect of flow rate.

For $Pe \leq 5$ in a fixed-bed reactor containing a immobilised enzyme with a kinetics reaction given by

Table 4
Experimental and predicted conversion values obtained for the axial dispersion model with the type III kinetics

u ($cm\,s^{-1}$)	Pe	k_L	$S_0 = 0.073$ M			$S_0 = 0.292$ M		
			Experimental	Predicted	Residuals[a]	Experimental	Predicted	Residuals[b]
0.034	12.66	0.0396	–	–	–	0.838	0.826	0.012
0.039	14.56	0.0419	0.936	0.911	0.025	0.796	0.781	0.015
0.049	18.36	0.0450	0.897	0.875	0.022	0.722	0.713	0.009
0.059	22.16	0.0478	0.855	0.836	0.019	0.630	0.647	−0.017
0.067	24.06	0.0500	0.816	0.805	0.011	–	–	–
0.078	27.86	0.0525	0.777	0.765	0.012	0.521	0.545	−0.024
0.097	35.46	0.0566	0.711	0.697	0.014	0.431	0.463	−0.032
0.119	45.12	0.0604	0.646	0.633	0.012	0.362	0.396	−0.034

[a] $S_e = 0.019$.
[b] $S_e = 0.024$.

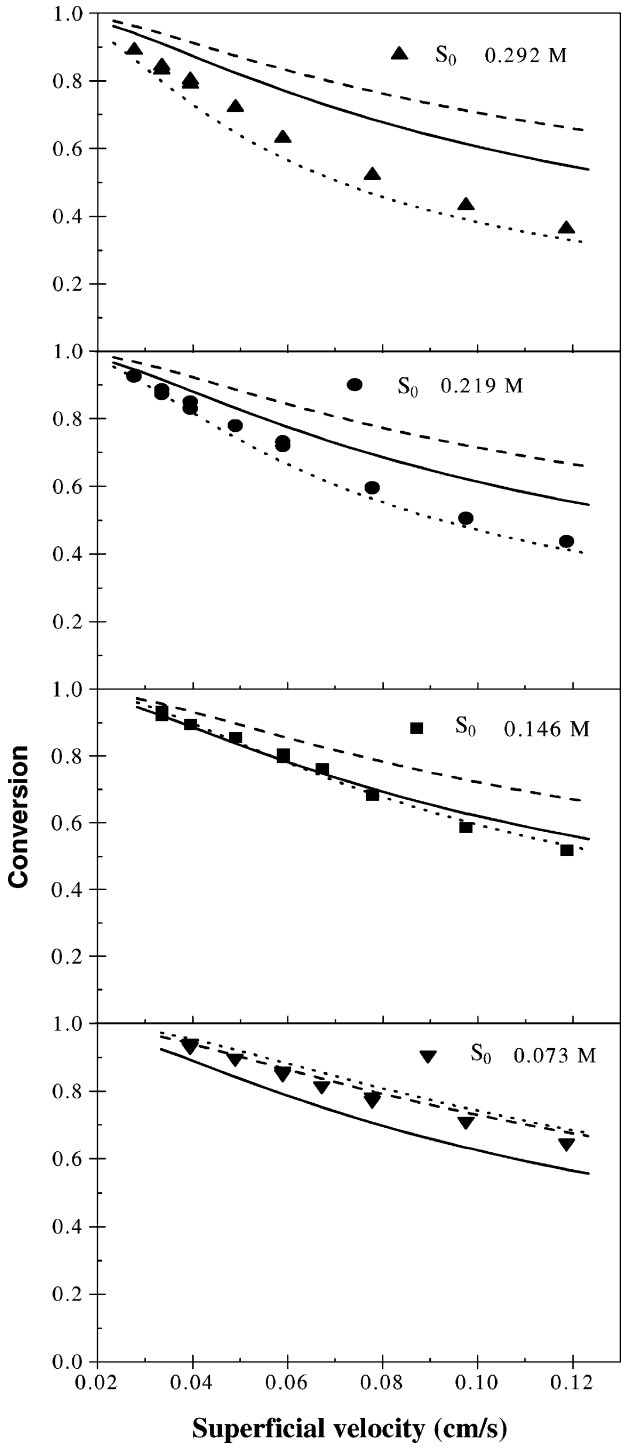

Fig. 2. Experimental data and calculated values with plug-flow model for kinetic equations: (—) type I; (– – –) type II; (· · ·) type III.

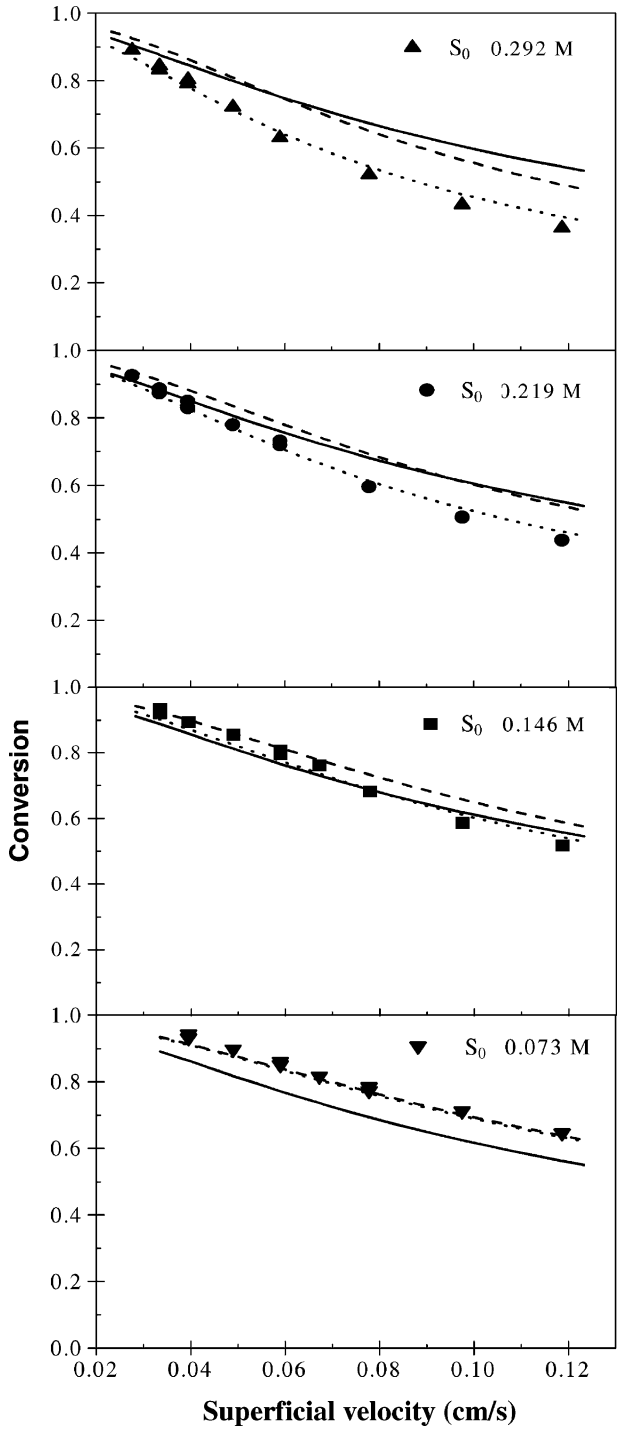

Fig. 3. Experimental data and calculated values with dispersion model for kinetic equations: (—) type I; (– – –) type II; (· · ·) type III.

Michaelis–Menten with product inhibition, the conversion values should not be significantly different after using a model that only considers plug-flow or the axial dispersion system [10], while for $20 < Pe < 100$, which is the most common situation in a tubular reactor, the axial diffusion in the model must be considered, in order to predict experimental results [19]. In the experimental assays *Pe* values changed from 12.7 to 45.1 according to superficial velocity. Therefore, for the range of *Pe* numbers used, the axial dispersion model predicted the experimental behaviour with higher accuracy than the plug-flow model (Table 5, Figs. 2 and 3). The results of this study not only provided numerical solutions but also a deep understanding of the reaction systems.

5. Conclusions

Models for predicting fixed-bed reactor behaviour with a immobilised enzyme were compared considering different kinetics. The correct kinetic equation showed higher influence on the prediction of reactor behaviour than the characteristics assumed of the flow distribution. Besides, experimental results predicted by the axial dispersion model, showed higher accuracy.

In this case, a model that considered different factors such as kinetics and hydrodynamic conditions solved by numerical methods using the parameters known for ideal systems predicted the experimental behaviour in all the concentration range with small error. This result is very important for reactor scaling up, without fitting previously the parameters at the working condition.

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Appendix A

(a) Expressions to α for different kinetics

Kinetics of first-order (I):

$$\alpha = \frac{k_L a}{(k' + k_L a)} \tag{A.1}$$

Kinetics of Michaelis–Menten (II):

$$\alpha = \frac{S + c_1 + \sqrt{(S + c_1)^2 + S c_2}}{2S} \tag{A.2}$$

where

$$c_1 = -K'_m - \frac{V'_{max}}{(k_L a)}, \qquad c_2 = 4K'_m$$

Kinetics of Michaelis–Menten with competitive inhibition by product (III):

$$\alpha = \frac{S c_3 + c_6 + \sqrt{(S c_3 - c_6)^2 + 4S(c_4 - c_5 S)}}{2S} \tag{A.3}$$

where

$$c_3 = K'_m + 1, \qquad c_4 = K'_m + \frac{K'_m}{k'_p} S_0,$$

$$c_5 = \frac{K'_m}{k'_p}, \qquad c_6 = c_4 + \frac{V'_{max}}{k_L a}$$

(b) Fit standard error

$$S_e = \sqrt{\frac{\sum_{j=1}^{n} (\hat{y}_j - y_j)^2}{n - m}} \tag{A.4}$$

where y_j is the y data value, $\hat{y}_j$ the estimated y value, n the total number of data points and m the number of coefficients in the equation.

(c) Coefficient of determination

$$r^2 = 1 - \frac{\sum_{j=1}^{n} (\hat{y}_j - y_j)^2}{\sum_{j=1}^{n} (y_j - \bar{y})^2} \tag{A.5}$$

where y_j is the y data value, $\hat{y}_j$ the estimated y value, $\bar{y}_j$ the mean of the y data values and n the total number of data points.

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