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A method for evaluating lactose hydrolysis in a fixed bed reactor with β-galactosidase immobilized on chitosan

Carlos R. Carrara, Amelia C. Rubiolo *

Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional del Litoral (UNL), Güemes 3450, 3000 Santa Fe, Argentina

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Abstract

A model for evaluating lactose hydrolysis with immobilized enzyme in a packed bed reactor was developed. It is assumed that the conditions were plug flow and Michaelis–Menten kinetics with product competitive inhibition. The effects on the reaction rate of the external mass transfer resistance were considered by estimating the total fluid mass transfer coefficient. Experimental results were determined using β-galactosidase of *Kluyveromices fragilis* immobilized on chitosan beads in a packed bed reactor under special conditions, in order to calculate the parameters for different flow ranges and substrate concentrations. The model with these values allowed us to fit the reactor behavior for a wider range of work.

Keywords: β-Galactosidase; Lactose; Reactor; Model

1. Introduction

An immobilized enzyme can be used over a longer period of time than an enzyme solution, giving the chance of working with a continuous system, because it is operated in packed columns. A commercially viable process that uses immobilized enzyme can be a better approach, taking into account cost and efficiency. A low cost support material combined with a good enzyme loading and a long working period are required to determine the viability of the system.

The packed bed reactor is more efficient because of the higher enzyme concentration by volume unit and small free volume. Furthermore, it is more suitable than a continuous-flow stirred tank reactor when product inhibition [1] is present in the enzyme reaction.

A great number of publications related to packed bed, immobilized enzyme reactors have described the development of different mathematical models that take into account the external mass transfer resistance. These models consider the simple Michaelis–Menten equation (Eq. (2) below) in the reaction kinetics and other simplifications; or a numerical solution of the equations for several kinetic expressions [3].

The objective of this work was to study lactose hydrolysis by β-galactosidase attached on chitosan beads in a packed bed reactor [4]. This involves the identification of an appropriated reactor model that describes both the kinetics of the hydrolysis reaction and the flow inside the reactor, to determine the lactose conversion.

1.1. Theoretical solutions

The mathematical expression for the substrate material balance of an isothermal packed bed reactor operating under steady state and plug flow conditions is

$$U\frac{dS}{dZ}=v \tag{1}$$

with the initial condition $S=S_o$ at $Z=0$. Here, U is the superficial velocity, S is the substrate concentration, Z is the length along the packed bed and v is the reaction velocity.

The kinetic system commonly accepted for lactose hydrolysis by β-galactosidase is the Michaelis–Menten expression with competitive inhibition by galactose [5], i.e.

$$v=-\frac{V_m' S_i}{K_m'[1+P/(k_i')]+S_i} \tag{2}$$

This equation considers the following intrinsic kinetics parameters: $V_m'=E^\circ W_{sop}k_2'$, K_m', k_i' and the substrate concentration (S_i) which is taken on the catalyst surface due to the enzyme immobilization being fixed on the support surface [6]. E° is the initial enzyme concentration and W_{sop} is the support weight.

* Corresponding author.

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The relationship between S and S_i is given only by the mass transfer in the liquid and the reaction rate. It is assumed that no partition effects exist [7], because the substrate has no net charge to modify the distribution between liquid and solid at the surface interface. We have

$$v = k_s a(S - S_i) \tag{3}$$

where k_s is the coefficient of mass transfer and a is the specific area of particles in the packed bed unit volume. Rewriting Eq. (1) and Eq. (2) with the dimensionless variable $\alpha = (S_i/S)$, substrate conversion $x = (S_o - S)/S_o$ and $P = (S_o - S)$, we obtain

$$UQ\frac{d(-x)}{dZ} = -\frac{\alpha(1-x)}{R+(1-x)(\alpha-G)} \tag{4}$$

with the initial conditions $x = 1$ at $Z = 0$.

The same dimensionless variables can be used with Eq. (2) and Eq. (3) to obtain

$$(1-\alpha) = \frac{1}{QH}\frac{\alpha}{R+(1-x)(\alpha-G)} \tag{5}$$

where

$$R = \frac{K_m'}{S_o}\left(1+\frac{S_o}{k_i'}\right)$$

$$Q = \frac{S_o}{V_m'}$$

$$G = \frac{K_m'}{k_i'}$$

$$H = k_s a$$

When Eq. (4) and Eq. (5) are solved simultaneously, we obtain

$$\frac{L}{UQ} = W_1 \ln Y + W_2(Y-1) + W_3(Y^2-1) + W_4(F_1 - F_0) + W_5 J_1 + W_6 J_2 \tag{6}$$

where

$$Y = (1-x) \tag{6a}$$

$$W_1 = \frac{D}{HQ} - 2R \tag{6b}$$

$$W_2 = 2G - 3 + \frac{2HR}{D} + \frac{1}{2D} \tag{6c}$$

$$W_3 = \frac{HQ}{2D}(1-2G) \tag{6d}$$

$$W_4 = \frac{c}{4bD} - \frac{1}{HQ} + \frac{HQ}{b}(2G-1) \tag{6e}$$

$$W_5 = D - 2HQR - \frac{c}{2HQ} - \frac{HQc}{2b}(2G-1) + \frac{4be-c^2}{8bD} \tag{6f}$$

$$W_6 = \frac{e^{1/2}}{HQ} \tag{6g}$$

$$F_0 = (b+c+e)^{1/2} \tag{6h}$$

$$F_1 = (bY^2+cY+e)^{1/2} \tag{6i}$$

$$J_1 = -\frac{1}{(-b)^{1/2}}\left\{\text{sen}^{-1}\left[\frac{2bY+c}{(c^2-4be)^{1/2}}\right] - \text{sen}^{-1}\left[\frac{2b+c}{(c^2-4be)^{1/2}}\right]\right\},\ b<0 \tag{6j}$$

$$J_1 = \frac{1}{b^{1/2}}\left[\ln\left(\frac{2b^{1/2}F_1+2bY+c}{2b^{1/2}F_0+2b+c}\right)\right],\ b>0 \tag{6k}$$

$$J_2 = \ln\left(\frac{1}{Y}\frac{2e^{1/2}F_1+cY+2e}{2e^{1/2}F_0+c+2e}\right) \tag{6l}$$

Here, we take $b = HQ(HQ-4M_2)$, $c = HQ(2M_1-D)$, $M_1 = HQR$, $M_2 = HQG$, $D = M_1 - M_2 + 1$ and $e = D^2$.

An algebraic complex expression was found after solving the equations to determine the substrate conversion, which was calculated by successive approximation with computational routines.

1.2. Estimation of film mass transfer coefficients

The external mass transfer coefficient k_s was estimated as a function of the superficial velocity U by

$$k_s = C'U^{(1-p)} \tag{7}$$

where

$$C' = Cd_p^{-p}D_s^{2/3}(\delta_s/\mu_s)^{(2/3-p)}$$

with d_p the catalyst diameter, D_s the substrate diffusion coefficient and, δ_s and μ_s the density solution and viscosity respectively. The parameters C and p are characteristic for each system, and were obtained by linear regression of log k_s vs. log U for each set of assays at equal initial substrate concentrations. This correlation was derived from the model of Chilton and Colburn [9] and from the flux equation, which are commonly used in studies of mass transfer in packed bed reactors [3,8].

The mass transfer coefficient k_s from experimental values was calculated for $R > [(1-x)(\alpha-G)]$ for high substrate conversion ($x > 0.73$) [2]. Solving Eq. (3) and Eq. (4) with this simplification, we obtain

$$k_s = \frac{1}{a}\left[-\frac{L}{U\ln(1-x)} - \frac{V_m'}{K_m'}\left(1+\frac{S_o}{k_i'}\right)\right]^{-1} \tag{8}$$

The specific area a of particles in the packed bed unit volume was estimated as

$$a=\frac{6(1-\epsilon)}{d_p}$$

where ϵ is the average void fraction [8].

1.3. Deactivation for immobilized enzyme

The deactivation rate as a function of the enzyme concentration for immobilized enzyme can be expressed as [10]

$$E^\circ \xrightarrow{k_d} E$$

where E° is the initial enzyme concentration (grams of protein per gram of support weight), E is the active enzyme concentration (grams of protein per gram of support weight) at time θ (h) and k_d is the deactivation constant (h^{-1}).

If the decreasing activity obeys a first-order reaction, and taking into account the catalyst period of use under continuous operation, then the deactivation process can be expressed

$$E=E^\circ \exp(-k_d\theta) \quad (9)$$

A set of assays was carried out at constant flow to determine the conversion values used to calculate the variation of E (active enzyme concentration) as a function of time using Eq. (6). Afterwards, Eq. (9) was applied in the form $\ln E/E^\circ$ vs. θ, and k_d was determined by linear regression.

2. Materials and methods

Lactozym 3000 (*Kluyveromices fragilis* β-galactosidase) was obtained from Novo (Denmark). This commercial preparation (0.035 g protein cm^{-3}) was used without further purification; it had an activity of 1.3×10^{-3} mol glucose s^{-1} per gram of protein (78.5 IU) at pH 6.86 (0.025 M potassium phosphate buffer), as determined using lactose as the substrate (0.146 M) at a temperature of 37 °C.

Kits for enzymatic determination with glucose were obtained from Wiener Lab (Argentina). Crab shell chitosan and sodium tripolyphosphate of practical grade were obtained from Sigma Chemical Co. (USA). All the other chemicals were of analytical grade and obtained from Mallinckrot or Merck (USA).

2.1. Preparation of immobilized enzyme

The β-galactosidase was immobilized on chitosan beads using glutaraldehyde, as described previously [4]. The beads had an average diameter d_p of 0.22 cm and a density of 1.102 g cm^{-3}. The value of E° determined was 0.021 g protein g^{-1} support weight. The kinetic constants were obtained previously [11] in a batch system from an experimental data series with different initial substrate concentrations, determined under negligible mass transfer conditions. The values used were $K_m' = 0.137$ M glucose, $k_i' = 0.234$ M and $k_2' = 1.3\ 10^{-2}$ M s^{-1} g^{-1} of protein.

Data of Weast and Melvin [12] and Perry and Chilton [13] were used in the equation

$$\text{constant} = D_s\mu_s/T$$

to modify the parameter values for temperature changes.

2.2. Packed bed reactor

A (14.0 cm × 1.2 cm) column with a water recirculation jacket and a heating water bath were used with immobilized enzyme beads as the isothermal packed bed reactor at 43 °C. Chitosan spherical particles with immobilized β-galactosidase were packed in the reactor column. The outlet product (glucose) concentrations were measured over a wide range of inlet feed flows and for four substrate concentrations. Lactose solutions with concentrations S_0 of 2.5%, 5.0%, 7.5% and 10.0% in 0.025 M KH_2PO_4 and 0.025 M Na_2HPO_4 buffer of pH 6.86 were pumped by a peristaltic pump at a constant flow (in the range 113.0–483.0 ml h^{-1}). The weight of the chitosan beads was 10.66 g and the average void fraction ϵ of the packed bed was 0.389.

The average substrate conversion for two or three samples was determined when the reactor was under steady state conditions. This procedure was repeated for every flow rate, considering that the steady state of each assay was reached after waiting the time required to pass through the reactor a solution volume equal to 10 times the reactor free volume (void fraction of reactor volume).

Assays were carried out to study the decreasing catalyst activity at a constant flow (225.0 ml h^{-1}) during a period of 1 h. The amount of glucose was measured, averaging four values for steady state conditions.

3. Results and discussion

Table 1 shows the experimental conversion values obtained for different inlet feed flows at four initial substrate concentrations for the reactor in the steady state. k_s values under conditions of different flow rates and substrate concentrations (given in Table 2) were obtained from the data for $x>0.73$ in Table 1 using Eq. (8). C and p from the correlation of Chilton and Colburn were determined using the k_s values calculated for each initial substrate concentration. Fig. 1 shows the k_s values determined and those estimated by the correlation of Chilton and Colburn for different superficial velocities. It was found that Eq. (7) could be used with an average p value; however, it should be considered with specific C values for each substrate concentration (Table 3) to calculate experimental values with small errors.

The average p value was similar to the value $p=2/3$ recommended [9] for the Reynolds number (Re) range from 0.0016 to 55 and the Schmid number (Sc) range from 165 to 70.000. The C value for the same Re and Sc ranges would

Table 1
Lactose conversion as a function of the initial concentration and superficial velocity

U (cm s^{-1})	Lactose conversion			
	$S_o = 2.5\%$	$S_o = 5.0\%$	$S_o = 7.5\%$	$S_o = 10.0\%$
0.028			0.925 ± 0.000	0.881 ± 0.013
0.033		0.918 ± 0.000	0.886 ± 0.012	0.844 ± 0.000
		0.931 ± 0.013	0.874 ± 0.000	0.832 ± 0.012
0.039	0.931 ± 0.007	0.894 ± 0.012	0.850 ± 0.012	0.803 ± 0.000
	0.925 ± 0.013	0.894 ± 0.012	0.837 ± 0.000	0.791 ± 0.012
0.049	0.887 ± 0.000	0.855 ± 0.000	0.789 ± 0.000	0.734 ± 0.000
0.059	0.839 ± 0.000	0.795 ± 0.011	0.745 ± 0.000	0.655 ± 0.000
	0.851 ± 0.012	0.807 ± 0.000	0.734 ± 0.010	
0.067	0.816 ± 0.000	0.762 ± 0.000		
0.078	0.772 ± 0.000	0.682 ± 0.019	0.595 ± 0.000	0.511 ± 0.000
	0.783 ± 0.011			
0.098	0.711 ± 0.000	0.586 ± 0.008	0.498 ± 0.007	0.421 ± 0.000
0.119	0.646 ± 0.009	0.518 ± 0.014	0.427 ± 0.000	0.344 ± 0.000

Table 2
Mass transfer coefficient as a function of Re

U (cm s^{-1})	$S_o = 2.5\%$		$S_o = 5.0\%$		$S_o = 7.5\%$		$S_o = 10.0\%$	
	Re	k_s ($\times 10^{-3}$ cm s^{-1})	Re	k_s ($\times 10^{-3}$ cm s^{-1})	Re	k_s ($\times 10^{-3}$ cm s^{-1})	Re	k_s ($\times 10^{-3}$ cm s^{-1})
0.028	0.84		0.79		0.73	0.569	0.67	0.452
0.033	1.01		0.95	0.654	0.88	0.586	0.81	0.498
				0.740		0.535		0.461
0.039	1.19	0.843	1.12	0.729	1.04	0.623	0.96	0.528
		0.791		0.729		0.572		0.492
0.049	1.48	0.855	1.39	0.830	1.29	0.642	1.19	0.541
0.059	1.78	0.871	1.67	0.812	1.55	0.719	1.43	
		0.942		0.875		0.675		
0.067	2.03	0.972	1.90	0.867	1.77		1.63	
0.078	2.35	0.997	2.21		2.05		1.89	
		1.066						

Sc = 1014.2–1605.9.

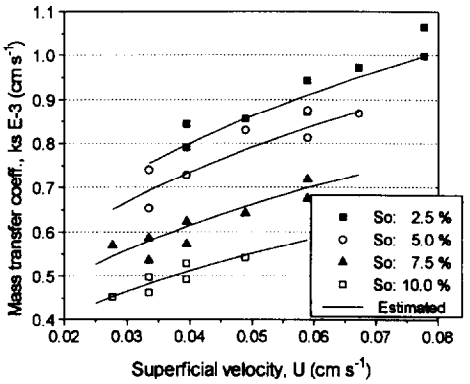

Fig. 1. Mass transfer coefficient values calculated using Eq. (8) with experimental data, and those estimated using Eq. (7).

be $C = 1.09/\epsilon = 2.80$, which is higher than the coefficient determined. The errors between the conversion values estimated with $C = 2.80$ in Eq. (6) and the experimental values were of the order of 20%–30% for higher inlet substrate concentrations and higher flows, while a maximum error of 6% was obtained for the same calculations using the C values that correspond to the conditions employed (Fig. 2).

The k_s value increased when the substrate concentration decreased; this behavior was found by Park [8]. The k_s values determined for different substrate concentrations were lower than the values recommended [14] for a packed bed reactor with a low flow rate ($Re < 1$). A possible reason why the model of Chilton and Colburn did not fit for all substrate concentrations could be that the diffusion coefficient of the lactose in water might be modified by the presence in the solution of buffer salts. Furthermore, the k_s value was calculated using Eq. (8), when the solution was changing its substrate concentration as a result of its hydrolysis. Consequently, the different rates of change of the solution properties for each concentration could affect the determination of C.

The validity of the simplification made when Eq. (7) was obtained for $x > 0.73$ can be analyzed in Fig. 3. Eq. (6) fitted all the conversion experimental values, while the simplification given by Eq. (7) could only fit the values for $x > 0.75$.

Table 3
C and p calculated to estimate the mass transfer coefficient k_s

	$S_o = 2.5\%$	$S_o = 5.0\%$	$S_o = 7.5\%$	$S_o = 10.0\%$	Mean
p	0.656	0.661	0.684	0.675	0.669 ± 0.005
C	2.29	2.20	1.95	1.72	2.040 ± 0.003

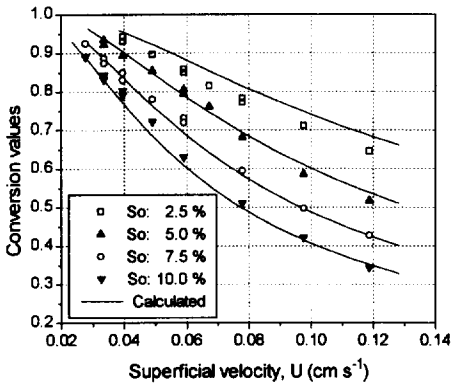

Fig. 2. Experimental conversion values and those calculated using Eq. (6), shown as a function of the superficial velocity.

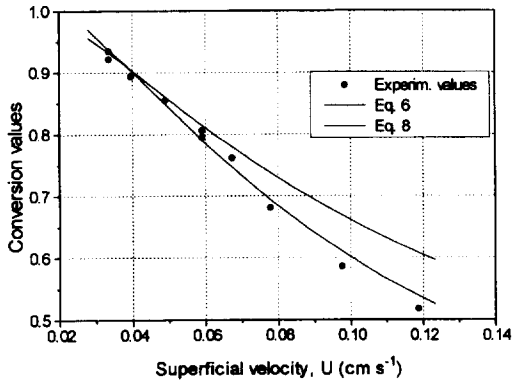

Fig. 3. Experimental values and those calculated using Eq. (6) and Eq. (8) for a substrate concentration of 5.0%.

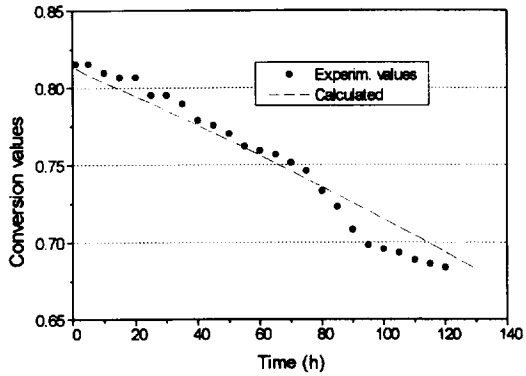

Fig. 4. Experimental conversion values and those calculated considering the decrease of catalyst activity with the working time.

The corresponding experimental substrate conversions were compared with those predicted by Eq. (6), using the calculated parameters for the system. The calculated and experimental values are shown in Fig. 2. This model could fit the packed bed reactor behavior under flow and substrate concentration changes. Therefore, greater deviation was found for smaller substrate concentrations.

The deactivation coefficient determined by linear regression was $k_d = 1.07 \times 10^{-2}\ h^{-1}$. Fig. 4 shows the experimental values and those determined using Eq. (6), with E values given by Eq. (9) taking into account the enzymatic activity loss. It was also found that the conversion value had decreased to half of its initial value after 80 h.

4. Conclusions

Exact determination of the behavior of a packed bed reactor was obtained considering steady state and plug flow conditions, along with the kinetics expression of Michaelis–Menten with competitive product inhibition. Estimation of the mass transfer coefficient was carried out and the parameter for the correlation of Chilton and Colburn was determined using the experimental results, which substituted in the model developed and fitted successfully for lactose hydrolysis with flow and substrate concentration changes.

Appendix A. Nomenclature

a	specific area of particles per unit packed bed volume (cm^{-1})
C	constant of Eq. (7)
d_p	bead diameter (cm)
D_s	substrate diffusion coefficient in water ($cm^2\ s^{-1}$)
E	active enzyme concentration (g protein g^{-1} support weight)
E°	initial enzyme concentration (g protein g^{-1} support weight)
k_2'	intrinsic specific rate constant ($M\ s^{-1}\ g^{-1}$ protein)
k_i'	intrinsic inhibition constant (M)
K_m'	intrinsic Michaelis constant (M)
k_d	deactivation rate constant (h^{-1})
k_s	mass transfer coefficient ($cm\ s^{-1}$)
L	reactor length (cm)
P	galactose concentration (M)
p	exponent of Eq. (7)
Re	Reynolds number
S	substrate concentration in the bulk (M)
Sc	Schmid number
S_i	substrate concentration in the catalyst surface (M)
S_o	substrate concentration at the reactor inlet (M)
T	temperature (K)

U superficial velocity through the packed bed (flow per section) (cm s^{-1})
v expression of reaction rate
V_m' intrinsic maximum reaction rate (M s^{-1})
W_{sop} weight of support (g)
x conversion of lactose
Z length along the packed bed (cm)

Greek letters

α S_i/S
ϵ void fraction of the packed bed
μ_s solution viscosity (g cm^{-1} s^{-1})
δ_s solution density (g cm^{-3})
θ time (h)

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