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Predicting the packed-bed reactor performance with immobilized microbial lactase

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Abstract

A mathematical model for several working conditions was numerically solved to study a fixed bed reactor with a biocatalyst. The effect of bed swelling, mass transfer limitations, axial dispersion, residence times and inlet lactose concentration were studied. A Michaelis–Menten kinetics with competitive product inhibition was used to represent the enzymatic reaction of the lactose hydrolysis by the β-galactosidase. Lactose conversion was used to determine the appropriate conditions for the immobilized enzyme reactor. The model predicted the experimental data with errors less than 2.5%.

Keywords: Packed-bed reactor; Lactose hydrolysis; Model

1. Introduction

The use of packed-bed reactors in biological processes would allow the application of new methodologies to transform an environmental problem, such as permeate whey elimination of dairy industries, in a commercial affair [1].

Lactose hydrolysis process with the appropriate enzyme favours the formation of a sweeter mixture, but it is economically feasible only with immobilized enzymes. This transformation has product inhibition. Therefore a tubular reactor has the advantage of a higher average reaction rate compared with a continuous stirred tank reactor because: (a) the inhibition effect decreases due to the low difference between substrate and product concentrations in the hole reactor, and (b) the enzyme loss is reduced due to the absence of collisions between biocatalyst particles and impeller and liquid shearing. Moreover, is also easier to operate besides to offer high efficiency and conversions [2].

Therefore, an isothermal packed-bed reactor was chosen for lactose hydrolysis by β-galactosidase entrapped into polysaccharide gels. Usually, these reactors are operated in the integral mode and the reactor model to describe their performance is only based on the plug-flow regime.

In many cases, for immobilized enzyme reactors, it is not possible to use the plug-flow model due to disturbing effects such as mass transfer limitations, axial dispersion and bypassing that can not be potentially suppressed and complex terms are required in the model. Therefore, a realistic analysis of the packed-bed enzymatic reactor should include some fundamental aspects of the process such as liquid-phase (external) and the solid-phase (internal) mass transfer, intrinsic kinetic parameters and reactor hydrodynamics [3]. These physical considerations determine a mathematical model representing more accurately the reactor behaviour as a function of operational conditions.

The objective of this work was to study the performance of immobilized enzyme reactor performing lactose hydrolysis at various operational conditions. Lactose conversion was used as performance criterion for the immobilized enzyme reactor.

2. Theory

2.1. Reaction kinetics

For enzymatic hydrolysis of lactose to glucose and galactose by β-galactosidase, the reaction kinetics was well defined as

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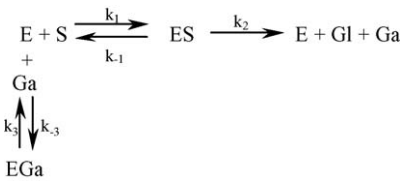
Michaelis–Menten with competitive inhibition by product [4,5]. The scheme of reaction mechanism can be expressed as:



(1)

where E is the β-galactosidase enzyme; S the lactose; ES the enzyme–substrate complex (β-galactosidase-lactose); Gl the glucose; Ga the galactose; EGa is the enzyme–product complex (β-galactosidase-galactose). Assuming that a briefly transient-state exists and that enzyme–substrate complex concentration is constant compared to substrate and product concentrations, the reaction rate for stationary-state can be expressed by:

$$\nu = \frac{\mathrm{d}[S]}{\mathrm{d}t} = -\frac{V_{\max}[S]}{\mathrm{Km}\left(1 + \frac{[\mathrm{Ga}]}{k_\mathrm{i}}\right) + [S]} \tag{2}$$

where $[S]$ is the substrate concentration (lactose) in the biocatalyst; $V_{\max} = k_2\,[E]_0$ the intrinsic maximum reaction rate; $[E]_0$ the concentration of active enzyme at initial time; $\mathrm{Km} = (k_{-1} + k_2)/k_1$ the intrinsic Michaelis–Menten constant; $k_\mathrm{i} = k_3/k_{-3}$ the intrinsic inhibition constant by product and [Ga] is the product concentration (galactose) in the biocatalyst, which was considered equal to $[S]_0 - [S]$, being $[S]_0$ the substrate concentration at initial time in the biocatalyst.

2.2. Activity loss and half-life of the immobilized enzymes

The activity loss of an immobilized enzyme can occur due to changes in its spatial structure or polypeptide chain ruptures produced by reaction conditions (pH, temperature, reaction medium ionic force, etc.), permanent blockade of enzyme active sites by present inhibitors or by links to support, steric and solvent effects, enzyme protein loss initially fixed to the support, etc. [6]. Even if these causes are individually manifested in different forms, for convenience a global evaluation is carried out.

Different mechanisms have been proposed to describe enzyme inactivation. The simplest and most used is one-stage first-order kinetics, which proposes the transition of a fully active native enzyme to a fully inactivated species in a single step. Such mechanism leads to a model of exponential decay, where the residual enzyme activity, A, at time t can be determined by the following equation:

$$A = \frac{[E]}{[E]_0} = \mathrm{e}^{-k_\mathrm{d}t} \tag{3}$$

where $[E]_0$ and $[E]$ are the concentrations of active enzyme at initial time and at any other time, respectively, and k_d is the deactivation rate constant, which follows the Arrhenius equation for the temperature dependence. Thermal inactivation is certainly more complex and series or parallel mechanisms have been proposed to describe it [6,7]. Models derived from such mechanisms contain a high number of parameters that are difficult to determine experimentally, and lead to a more difficult mathematical approach.

Therefore, still when this approach is considered an oversimplification especially in the case of immobilized enzymes; it can be used as a first one to improve isothermal reactor models. When only the thermal deactivation is considered, a simpler and more convenient form for determining the biocatalyst activity loss during continuous operation can be defined through the operational stability concept. The operational stability of a biocatalyst is commonly estimated by the apparent half-life, which is the elapsed time at which the initial catalytic activity is reduced to half [8].

Biocatalyst stability is a fundamental aspect in reactor performance. Thus, many researchers focused their work to investigate the deactivation of the immobilized β-galactosidase [9,10]. The half-life and the thermal deactivation for immobilized β-galactosidase at various temperatures [11,12] and at these operational conditions considering modulation effects by substrates and products, which certainly play a role during catalysis [13,14], were also investigated. Moreover, recently, some papers reports on the operational stability of the entrapped β-galactosidase [15,16].

2.3. Reactor model

The mathematical model for the substrate concentration profile in the liquid stream (b) of an isothermal packed-bed reactor of a length Z, operating in steady state, is the model often used [17]. Mass balance equations can be written in the following form:

$$D_z \frac{\partial^2[S]^b}{\partial z^2} + D_r\left(\frac{\partial^2[S]^b}{\partial r^2} + \frac{1}{r}\frac{\partial[S]^b}{\partial r}\right) - u_z\frac{\partial[S]^b}{\partial z} = \nu \tag{4}$$

where D_z and D_r are the axial and radial dispersion coefficients, respectively, z and r the axial and radial coordinates, respectively and u_z is the superficial fluid velocity in the reactor.

Usually, the influence of axial dispersion is more important than the radial one; and for packed-bed reactors with large length/diameter ratios, radial dispersion can be rejected. In this case, Eq. (4) can be reduced to:

$$\frac{D_z}{u_z}\frac{\mathrm{d}^2[S]^b}{\mathrm{d}z^2} - \frac{\mathrm{d}[S]^b}{\mathrm{d}z} = \frac{\nu}{u_z} \tag{5}$$

with boundary conditions:

$$z = 0^+ \quad [S]^b - [S]_0^b = \frac{D_z}{u_z}\frac{\mathrm{d}[S]^b}{\mathrm{d}z} \tag{6}$$

$$z = Z \quad \frac{\mathrm{d}[S]^b}{\mathrm{d}z} = 0 \tag{7}$$

where $[S]_0^b$ is the substrate concentration at initial time in the liquid stream.

On the other hand, for the proposed kinetic mechanism, the reaction rate expression considering that the utilized biocatalyst weight allows to obtain the initial quantity of immobilized

enzyme $[E]_0^i$, and that $[\mathrm{Ga}] \cong [\mathrm{Gl}] = [S]_0^b - [S]^b$, results:

$$R_S = -v = \frac{V_{max}[S]}{\mathrm{Km}\left(1+\frac{[\mathrm{Ga}]}{k_i}\right)+[S]} = \frac{[E]_0^i\, e^{-k_d t} k_2 [S]}{\mathrm{Km}\left(1+\frac{[S]_0^b-[S]^b}{k_i}\right)+[S]} \quad (8)$$

Taking into account that there is not a conformational change in the enzyme during the entrapment method used for the immobilisation, Km (Michaelis–Menten constant) and k_i (inhibition constant) are the values corresponding to the free enzyme. The main effect on the reaction rate is the intra-particle resistance, that is, the substrate difficulty to diffuse towards the active enzyme site. For this reason, coupling by the corresponding equations, kinetic parameters of the free enzyme with the effective diffusive values, kinetics parameters do not be restrictions to be able adjust the biocatalysts behaviour to different conditions of reactors.

Replacing Eq. (8) into Eq. (5), and after using dimensionless variables, $C^* = [S]^b/[S]_0^b$, $C = [S]/[S]_0^b$, $Pe = u_z Z/D_z$ and $x = z/Z$, equations can be written as follows:

$$\frac{[S]_0^b}{Pe}\frac{d^2C^*}{dx^2} - \frac{dC^*}{dx} - \frac{Z}{u_z}\frac{[E]_0^i\, e^{-k_d t} k_2 C}{\mathrm{Km}\left[1+\frac{[S]_0^b(1-C)}{k_i}\right]+[S]_0^b C} = 0 \quad (9)$$

To solve this equation, the dimensionless substrate concentration inside catalyst spherical particle, C, should be known; therefore, a mass balance considering diffusion and chemical reaction in steady state was considered:

$$\frac{1}{r^2}\frac{d}{dr}\left(r^2 D_S \frac{d[S]}{dr}\right) = R_S \quad (10)$$

where D_S is the effective diffusion coefficient of the substrate (lactose). The boundary conditions are:

$$r = 0 \quad \frac{d[S]}{dr} = 0 \quad (11)$$

$$r = R \quad \frac{d[S]}{dr} = \frac{k_l}{D_S}([S]^b - [S]) \quad (12)$$

where k_l is the external mass transfer coefficient. Using the dimensionless variables:

$$y = \frac{r}{R};\quad \Phi^2 = \frac{V_{max}R^2}{\mathrm{Km}\,D_S};\quad \gamma = \frac{[S]_0^b}{\mathrm{Km}};\quad \gamma_2 = \frac{[S]_0^b - [S]}{k_i}$$
$$\gamma_2' = \frac{[S]_0^b}{k_i};\quad \varphi = \frac{u_z R^2}{D_S Z};\quad \varphi_2 = \frac{u_z R^2 [S]_0^b}{Pe D_S Z};\quad Sh = \frac{k_l R}{D_S} \quad (13)$$

Eq. (9) resulting:

$$\varphi_2\frac{d^2C^*}{dx^2} - \varphi\frac{dC^*}{dx} = \frac{\Phi^2 C}{1+\gamma C+\gamma_2'(1-C)} \quad (14)$$

with the following boundary conditions:

$$x = 0 \quad C^* = 1 + \frac{\varphi_2}{\varphi[S]_0^b}\frac{dC^*}{dx} \quad (15)$$

$$x = 1 \quad \frac{dC^*}{dx} = 0 \quad (16)$$

And Eq. (10) resulting:

$$\frac{d^2C}{dy^2} = \frac{\Phi^2 C}{1+\gamma_2+\gamma C} \quad (17)$$

with the following boundary conditions:

$$y = 0 \quad \frac{dC}{dy} = 0 \quad (18)$$

$$y = 1 \quad C = 1 - \frac{1}{Sh}\frac{dC}{dy} \quad (19)$$

The method of orthogonal collocation with six internal points was employed to solve the equation system (14)–(19) [18]. This method is based on expanding the variables C^* and C in terms of orthogonal x and y, respectively, using a series of known functions Se_j to obtain an approximate solution in the x (0,1) and y (0,1) domains.

The series Se_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 right hand side 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 Se_j are the exact solution with a weighted residual equal zero. Then, the unknown coefficients q_i of the polynomials can be calculated for those 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.

Using $n = 6$ and weighting function $W = 1$ for each system, the collocation matrixes A and B were generated. Eqs. (14)–(19) in this form can be written:

Reactor fluid phase:

For $j = 1$:

$$x = 0 \quad C_j^* = 1 + \frac{\varphi_2}{\varphi[S]_0^b}\sum_{k=1}^{8} A_{kj}^* C_k^* \quad (20)$$

For $j = 2, \ldots, 7$:

$$\varphi_2\sum_{k=1}^{8} B_{kj}^* C_k^* - \varphi\sum_{k=1}^{8} A_{kj}^* C_k^* = \frac{\Phi^2 C_j}{1+\gamma C_j+\gamma_2'(1-C_j)} \quad (21)$$

For $j = 8$:

$$x = 1 \quad \sum_{k=1}^{8} A_{kj}^* C_k^* = 0 \quad (22)$$

Inside catalyst spherical particle:

For $j = 1$:

$$y = 0 \quad \sum_{k=1}^{8} A_{kj} C_k = 0 \quad (23)$$

For $j = 2, \ldots, 7$:

$$\sum_{k=1}^{8} B_{kj} C_k^* = \frac{\Phi^2 C_j}{1+\gamma_2+\gamma C_j} \quad (24)$$

For $j = 8$:

$$y = 1 \quad C_j = 1 - \frac{1}{Sh}\sum_{k=1}^{8} A_{kj} C_k \tag{25}$$

Eqs. (20)–(25), 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, it is not necessary to know the concentration profile inside the reactor, and only it is important to calculate the outlet flow. Therefore Eqs. (20)–(25) were solved to obtain the $[S]^b$ value at the reactor end for different flow velocities.

3. Materials and methods

3.1. Enzyme

The microbial lactase enzyme from *Kluyveromyces fragilis* (Lactozym 3000 L, Novo Nordisk A/S, Denmark) was used in this study. The initial activity of enzyme was determined immediately before their use and compared with the original value in microbial lactase unit (LAU). One LAU is defined as the quantity of enzyme that liberates 1 μmol of glucose per min at 37 °C and pH 6.5, using lactose 4.7% (w/w) as substrate. During the whole study, enzyme extract was stored in a refrigerator at 4 °C, and activity loss was not observed. The physicochemical characteristics of enzyme are shown in Table 1.

3.2. Hydrocolloids

Commercial hydrocolloids were used for enzyme entrapment. Sodium alginates, Keltone L.V. (low viscosity) and Keltone H.V. (high viscosity), were from Kelko (Chicago, USA) and Gelacid C-3 κ-carrageenan was from Biotec S.A. (Buenos Aires, Argentina).

3.3. Lactose solution

Lactose (reagent grade, Mallinckrodt, St. Louis, USA) was dissolved in phosphate buffer of pH 6.85 preparing four different lactose solutions 2.5–5.0–7.5–10.0% (w/v), which were used as substrate. The solution also contained potassium sorbate 0.1% (w/v) to prevent microbial growth in the reactor for long-term operation study.

3.4. Enzyme immobilization

A solution contained 2.4% (w/v) high viscosity sodium alginate, 0.5% (w/v) κ-carrageenan and 12.0% (v/v) β-galactosidase was prepared into a stirred glass container at room temperature. The solution composition was previously determined to ensure a high enzyme load and K^+ incorporation with a low viscosity of the solution [19].

The biocatalyst beads were obtained by forcing the mixture drop-wise through a needle at a rate of 30 drops/min, into a solution of calcium chloride 2.0% (w/v) (reagent grade, Mallinckrodt, St. Louis, USA) and potassium chloride 4.0% (w/v) (reagent grade, Mallinckrodt, St. Louis, USA). The droplet forms gel spheres of approximately 2.4 ± 0.2 mm diameter, entrapping the enzyme in a three-dimensional lattice of ionically cross-linked alginate and carrageenan.

Table 1
Physicochemical properties of used enzyme

Property	Lactozym 3000 L
Physical state	Liquid
Density (g/ml)	1.20
Initial activity (LAU/ml)	3000
Protein concentration (mg/ml)	35.09

The beads were kept in the solution for about 1 h to allow K^+ and Ca^{2+} ions diffuse into the beads and the gel strength increases. The beads were removed from the solution, washed and stored overnight at 4 °C in distilled water before using.

3.5. Determination of protein

Protein was quantified using a Proti-2 kit for total protein determination (Wiener Lab, Rosario, Santa Fe, Argentina) followed by absorbance measurement at 495 nm in a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY, USA) [20]. Hydrolysed casein (protein 13.6%, w/w, previously determined by Kjeldhal method) was used as standard. Measurements were performed in duplicate.

3.6. Estimation of entrapped protein in biocatalyst beads

The quantity of entrapped enzyme per gram of support was calculated by subtracting the protein quantity remained in the calcium chloride and potassium chloride solution after the immobilization step from the initial protein quantity presented in the hydrocolloid solution before gelling.

3.7. Determination of glucose

Glucose concentration was quantified using a kit for enzymatic glucose determination (Wiener Lab, Rosario, Santa Fe, Argentina) followed by absorbance measurement at 505 nm in a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY, USA). Measurements were performed in duplicate.

Concentrations of galactose and lactose were obtained from glucose concentration by assuming that 1 mol of glucose and 1 mol of galactose were formed from 1 mol of hydrolyzed lactose.

3.8. Determination of kinetic constants of enzyme

Taking into account that the entrapment method does not produce important alterations on the three-dimensional structure of the enzyme, it was considered that inherent kinetics parameters of the entrapped enzyme are similar to the intrinsic kinetics parameters of the free enzyme and modifications due to the difficulty to diffuse at the reaction site are taken in account by introducing this effect in the model.

The determination of the kinetics constants was carried out in a batch stirred reactor, without mass transfer effect, containing 20 ml of lactose solution of 2.5–5.0–7.5 and 10.0% (w/v), at 37 °C and pH 6.85, and using 20 and 40 μl of the enzyme. The free glucose concentration due to the lactose hydrolysis reaction was determined at different times. Measurements were performed in duplicate.

The integral method was used to obtain the kinetics parameters [4]. Eq. (2), considering that $[Ga] \cong [S]_0^b - [S]^b$, was expressed in conversion terms. The non-linear regression of values of X_S obtained for different values of $[S]_0^b$, was carried out using *E–Z* Solve® software. The values of Km, k_2 and k_i obtained were 98 ± 1 mmol l^{-1}, 11.4 ± 0.2 mmol l^{-1} min^{-1} mg^{-1} and 177 ± 2 mmol l^{-1}, respectively.

3.9. Reactors

A glass vessel (4.50 cm length × 3.86 cm diameter), with agitation and with a jacket for water recirculation at 37 °C, was used as a stirred batch reactor to determine kinetic constants of free enzyme.

A glass column (34.0 cm length × 1.47 cm diameter), with a jacket for water recirculation, was used with the biocatalyst as the isothermal packed-bed reactor.

3.10. Biocatalyst behaviour in a continuous system

A quantity of 39.0 g of fresh biocatalyst was loaded into the reactor, yielding a bead void fraction of 0.375. The reactor and biocatalyst characteristics are presented in Table 2.

The performance of immobilized microbial lactase in the packed-bed reactor was analyzed operating at 37 °C for long time periods. To determine the effect of substrate concentration on reactor productivity, lactose solution

Table 2
Characteristics of reactor and biocatalyst employed into assays

Reactor sizes	
Length (cm)	34.00
Diameter (cm)	1.47
Cross section (cm^2)	1.70
Total volume (cm^3)	57.80
External surface/reactor volume unity (a_e, cm^{-1})	2.55
Bed characteristics	
Bed diameter (cm)	0.24
Bed density (g cm^{-3})	1.08
Total biocatalyst weight in reactor (g)	39.0
Total biocatalyst volume in reactor (cm^3)	36.12
Free volume (cm^3)	21.68
Porosity (ε)	0.375

(2.5–10.0%, w/v) at pH 6.85 was introduced to the reactor at a flow rate of 250 ml h^{-1} with a peristaltic pump through the lower inlet part. The effect of flow rate on reactor performance was studied by varying the flow rate in the range of 100–500 ml h^{-1}. Initial glucose concentration values were measured at the end of the reactor after 20 min, considering that before this time the steady state was reached. The performance assays were carried out until the biocatalyst activity decreased to the 50% of the initial activity.

3.11. Gel swelling effect

It was determined in a previous work [19], using an empirical correlation:

$$\Delta R = 1.323 \times 10^{-5} t \frac{(1-\psi)^3}{(1+\psi)^2} \tag{26}$$

being:

$$\psi = \frac{8.483 \times 10^{-2}}{1 + Re_p} \tag{27}$$

where Re_p is the Reynolds number based on the particle radius and t is the reactor operation time.

3.12. Determination of biocatalyst stability

To determine the biocatalyst stability, a complete load of fresh biocatalyst was tested with a 250 ml h^{-1} flow of 5.0% (w/v) lactose solution at pH 6.85 in a packed-bed reactor operating 37 °C during 24 periods of 1 h each times. The biocatalyst deactivation constant k_d was calculated with obtained data according to Eq. (3).

3.13. Estimation of external mass transfer coefficient

For the prediction of mass transfer coefficient of lactose, the correlation proposed by Chilton and Colburn was used [21]. For a biocatalyst of diameter D:

$$k_l = \frac{1.09}{\varepsilon}\left(\frac{D_S}{D}\right)^{2/3} u_z^{1/3} \tag{28}$$

This correlation is useful to predict appropriately the value of mass transfer coefficient when $1.6 \times 10^{-5} < Re < 55$ and $165 < Sc < 70600$. Moreover, the specific area of the spherical catalyst particle, a, was calculated by:

$$a = \frac{6}{D}(1-\varepsilon) \tag{29}$$

3.14. Determination of axial dispersion coefficient

The axial dispersion coefficient (D_z) was estimated experimentally with the method used by Levenspiel [22], measuring the longitudinal spreading of the tracer concentration (glucose) in the output stream $R(t)$ during the period of time (t) that it was detected. The solution of transient equation for the dispersed model has a Gaussian distribution function. The relationship between the variance of the concentration versus the dimensionless time curve and the Peclet number (Pe) is:

$$\sigma^2 = \frac{2}{Pe\left[1 - \frac{1}{Pe(1-e^{-Pe})}\right]} \tag{30}$$

The signal for the outlet pulse was obtained with a differential refractometer. The area under the curve of response signal versus time was obtained by integration to determine σ^2, which was used to calculate Pe and then D_z [23]. The average $D_z = 0.56$ cm^2/s was experimentally obtained.

3.15. Estimation of substrate diffusion coefficient

The diffusivity of lactose at infinite dilution in water at 25 °C is $D_{Saq} = 5.21 \times 10^{-6}$ cm^2/s. The effective substrate diffusion constant in the gel is expected to be smaller than the mentioned value for infinitely diluted solution, mainly due to the sterical hindrance of the random movement of the substrate by the gel matrix [24,25]. Thus, the effective diffusion coefficient of lactose into biocatalyst was calculated according to Axelsson et al. [26]. This method proposed to calculate the value, considering the restriction factor due to solid fractions as a partition factor. The final equation is:

$$D_S = D_{S_{aq}} \frac{(1-\varphi)^3}{(1+\varphi)^2} \tag{31}$$

being:

$$\varphi = \frac{0.2387 f_i}{D^3} \tag{32}$$

where the constant f_i is an experimental factor according to proposed by Mackie and Meares, which value is 6.14×10^{-4}.

3.16. Data analysis

Experimental values of the lactose conversion in the continuous system were compared with theoretical values predicted using a program written in Matlab® language for the same reaction experimental conditions using a fixed-bed reactor model with axial dispersion, operating in steady state with a kinetics reaction for the immobilized enzyme of Michaelis–Menten with product inhibition. The program considered gel swelling and biocatalyst deactivation effects.

4. Results and discussion

The quantity of protein retained by the immobilization process was 10.5 mg of proteins per gram of biocatalyst.

Lactose conversion values obtained during kinetics assays performed in the batch reactor with 20 and 40 μl of the enzyme and with initial lactose concentrations of 2.5, 5.0, 7.5 and 10% are given in Tables 3 and 4. These values were used to determine by non-linear regression of X_S versus $[S]^b$ results, the following values for the intrinsic kinetic constants: Km = 98 ± 1 mmol l^{-1}; $k_2 = 11.4 \pm 0.2$ mmol l^{-1} min^{-1} mg^{-1}; $k_i = 177 \pm 2$ mmol l^{-1}.

The biocatalyst behaviour was study for different operational conditions in the fixed-bed reactor considering steady state. Fig. 1 shows the theoretical results of average bed diameter during the operation for different flow conditions obtained by Eq. (26). As it can be observed, the flow rate effect on the bed swelling could be important when the reactor operation time is long. After operating 100 h, increments of

Table 3
Lactose conversion values obtained with 20 μl of enzyme in a stirred batch reactor

Time (min)	X_S (%)			
	2.5	5.0	7.5	10.0
2	0.0000	0.0000	0.0000	0.0000
4	0.0721	0.0482	0.0345	0.0263
6	0.1401	0.0901	0.0669	0.0450
8	0.2112	0.1315	0.1005	0.0777
10	0.2806	0.1724	0.1369	0.1054
15	0.3387	0.2205	0.1695	0.1279
20	0.4812	0.3241	0.2432	0.1914
25	0.5709	0.4017	0.3012	0.2512
30	0.6517	0.4682	0.3611	0.3075

Table 4
Lactose conversion values obtained with 40 μl of enzyme in a stirred batch reactor

Time (min)	X_S (%)			
	2.5	5.0	7.5	10.0
2	0.0000	0.0000	0.0000	0.0000
4	0.1995	0.1422	0.1050	0.0800
6	0.3560	0.2639	0.2043	0.1633
8	0.4735	0.3778	0.2965	0.2347
10	0.5887	0.4822	0.3811	0.2909
15	0.6712	0.5699	0.4506	0.3502
20	0.8024	0.7112	0.5893	0.4810
25	0.8931	0.8124	0.6811	0.5817
30	0.9462	0.8796	0.7705	0.6600

47.1, 51.7 and 53.1% in the bed diameter were obtained for flow rates of 100, 300 and 500 ml h^{-1}, respectively.

For that operation time, the bead swelling affects the bed void fraction in the reactor and, therefore, the superficial velocity of the fluid into the reactor, as it can be observed in Fig. 2. Due to the proportionality between the bed void fraction in the reactor and the bead diameter, after operating 100 h, the superficial velocity was increased in the same way than the bead diameter for each flow rate.

Theoretical results obtained for different flow conditions after considering the variation of the external mass transfer of lactose by the product of Eqs. (28) and (29) are presented in Fig. 3, where it is observed that the effect of the operating time was not linear. After 100 h, increments in the external mass transfer of lactose of 27.6, 28.6 and 28.9% were obtained for flow rates of 100, 300 and 500 ml h^{-1}, respectively.

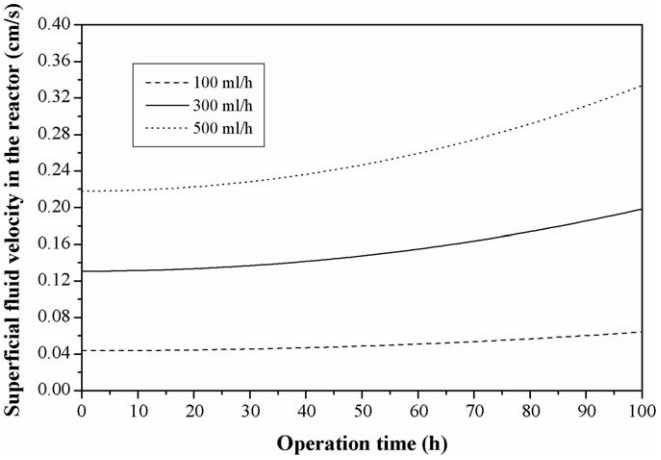


Fig. 2. Theoretical prediction for the superficial velocity into packed-bed reactor with the operation time.

The theoretical prediction for the effective diffusion coefficient of lactose into biocatalyst can be observed in Fig. 4. The effective diffusion coefficient of lactose into biocatalyst increased with operation time due to the bed swelling. After 100 h of reactor operation, the effective diffusion coefficient of lactose into biocatalyst increased 34.3, 35.9 and 36.4% for flow rates of 100, 300 and 500 ml h^{-1}, respectively. Also an average value of $D_z = 0.56$ cm^2/s was experimentally obtained for the axial dispersion coefficient.

Due to the gel swelling is non-linear and it depends on the agitation and the operation time of the reactor, the biocatalyst deactivation by protein loss is dependent on flow condition [16]. Nevertheless, in the analysed operation conditions in the packed-bed reactor, this factor was very small. Lactose conversion values obtained in packed-bed reactor feeding with 250 ml h^{-1} of 5.0% (w/v) lactose solution for different operation time are shown in Table 5.

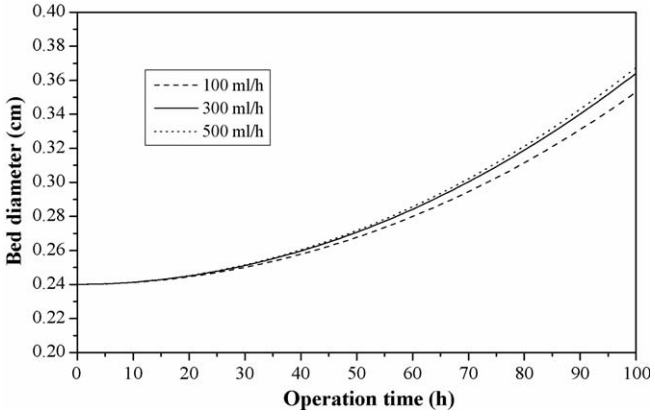


Fig. 1. Theoretical prediction for the variation of the average bed diameter with the operation time at different flow conditions.

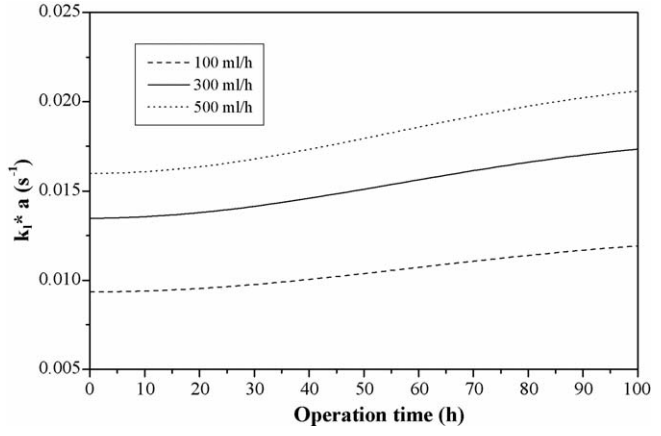


Fig. 3. Theoretical prediction for the variation of the external mass transfer of lactose during the operation time for different flow conditions.

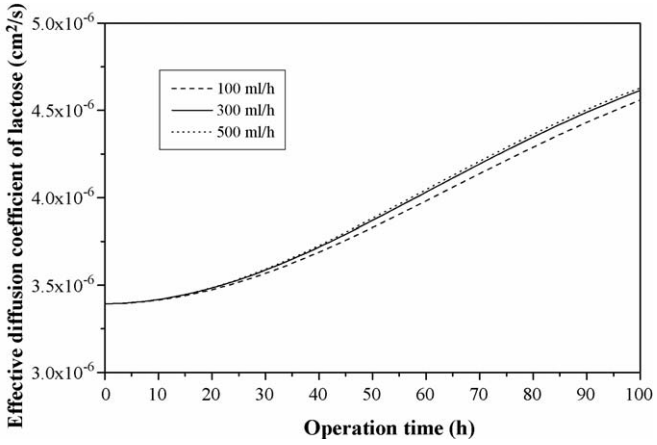


Fig. 4. Theoretical prediction for the effective diffusion coefficient of lactose into biocatalyst during the reactor operation.

During assays carried out to determine the deactivation effect, a k_d value of 6.40×10^{-3} h^{-1} was obtained ($r^2 = 0.996$). In this operation conditions, the biocatalyst have an apparent half-life of 108 h. In a previous work [19] it was determined the activity of β-galactosidase in solution and in the presence of mono and divalent ions and it was found that monovalent ions, in particular K^+, had a positive effect on the enzyme activity. κ-Carrageenan forms gel with K^+ ions, therefore activity would be increased due to the presence of the K^+ ion in the support coupling with κ-carrageenan gel. Unfortunately carrageenan gels are very weak and break easily, thus, precluding their use as supporting matrix for immobilized enzymes. However, if a mixture of gelled hydrocolloids is used as the supporting matrix, they can act synergistically with one gel favouring the enzymatic reaction (K^+-κ-carrageenan) and the other (Ca^{2+}-alginate) providing the necessary mechanical strength.

Initial lactose conversion values obtained in packed-bed reactor operating at different flow rates and with different initial lactose concentrations are in Table 6 where values show that higher lactose conversion values can be obtained at lower superficial velocities.

Table 5
Lactose conversion values obtained in the packed-bed reactor feeding with 250 ml h^{-1} of 5.0% (w/v) lactose solution at different operation time

Time (h)	X_S	Time (h)	X_S
0	0.6350	13	0.5840
1	0.6320	14	0.5780
2	0.6290	15	0.5750
3	0.6260	16	0.5720
4	0.6220	17	0.5710
5	0.6160	18	0.5650
6	0.6130	19	0.5620
7	0.6100	20	0.5590
8	0.6030	21	0.5560
9	0.6000	22	0.5525
10	0.5970	23	0.5460
11	0.5940	24	0.5430
12	0.5910		

Table 6
Lactose conversion values obtained in the packed-bed reactor at initial operation time

Superficial velocity (cm/s)	X_S (%)			
	2.50	5.00	7.50	10.00
0.0436	0.9050	0.8400	0.7900	0.7100
0.0871	0.7810	0.6700	0.6000	0.5000
0.1307	0.6800	0.5500	0.4500	0.3500
0.1743	0.5850	0.4400	0.3600	0.2700
0.2179	0.5100	0.3750	0.3000	0.2100

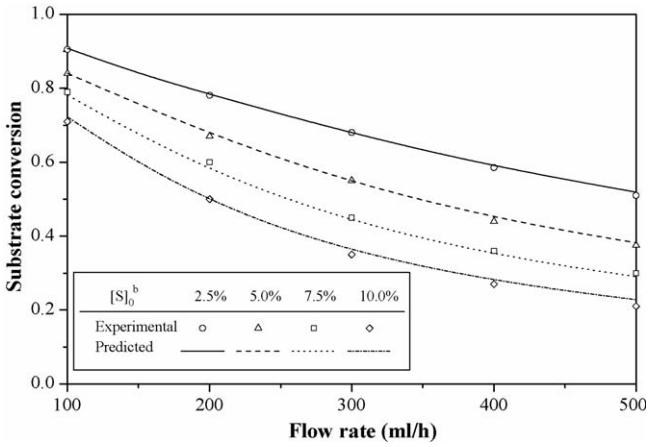


Fig. 5. Theoretical prediction and experimental data for conversion obtained for the same reaction experimental conditions.

Theoretical prediction and experimental data for conversion obtained for the same reaction experimental conditions are shown in Fig. 5, resulting that for the analysed flow-rate range, the theoretical model predicted the experimental behaviour with a smaller error, less than 2.5%.

Fig. 6 shows simulated variations of substrate conversion for different axial positions into packed-bed reactor feeding with a lactose solution of 5.0% (w/v). For the flow rate of 100 ml h^{-1}, a conversion value higher than 55% of final value was obtained

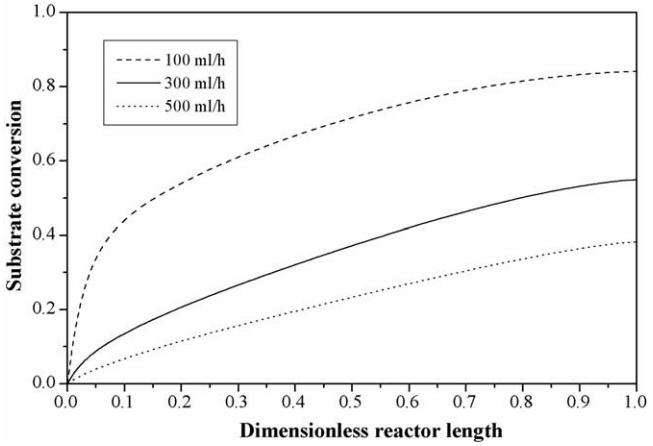


Fig. 6. Simulated variations of substrate conversion for different axial positions into packed-bed reactor feeding with a lactose solution of concentration equal to 5.0% (w/v).

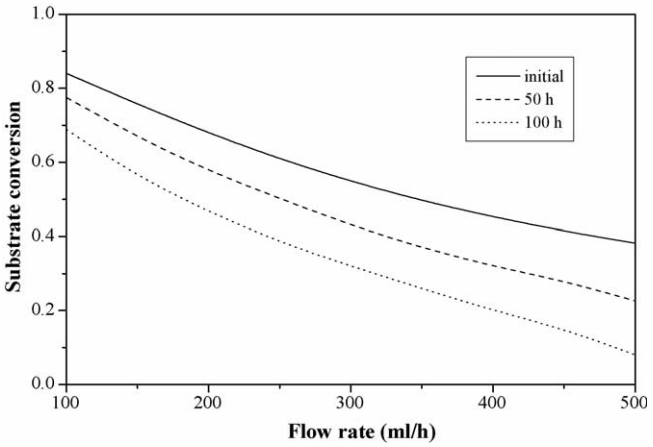


Fig. 7. Simulated variations of substrate conversion with the operation time, feeding the fixed-bed reactor with a 5.0% (w/v) lactose solution.

into first 10% of the total reactor length, while these values only reached approximately a 25% and 18% for 300 and 500 ml h^{-1}, respectively.

Fig. 7 shows simulated variations of substrate conversion with the operation time, feeding the fixed-bed reactor with a 5.0% (w/v) lactose solution. As the operation time was increased, an increase in the flow rate produces a bigger effect on the biocatalyst deactivation, being obtained therefore, a smaller conversion value. Thus, the reactor operation conditions for maintaining constant conversion, predicted using numerical solutions for the reactor model with the deactivation constant, is observed in Fig. 8 for a substrate concentration of 5.0% (w/v). For a conversion of 80%, the initial flow rate, $F_{(0)}$, was approximately 124.4 ml h^{-1}; for a conversion of 60%, the initial flow rate was approximately 257.3 ml h^{-1} and for a conversion of 40%, the initial flow rate was approximately 472.2 ml h^{-1}. Moreover, relating the operative flow rate at any time to the initial flow rate value for each constant conversion value, a small difference between the different values was obtained. This difference can be explained by the combined effect of the gel swelling on the superficial velocity of fluid into reactor and the mass transfer parameters.

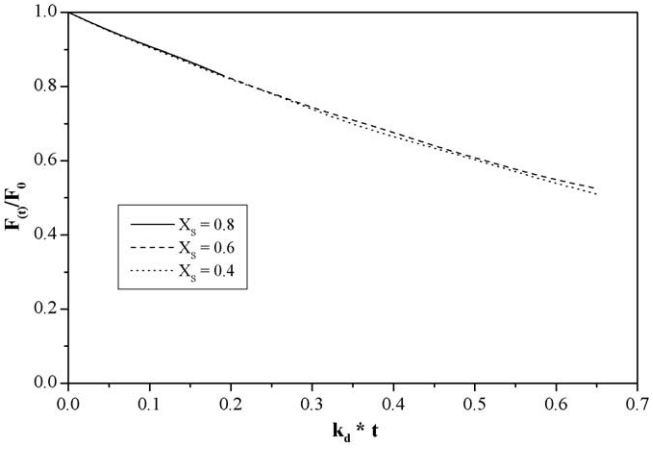


Fig. 8. Reactor feed flow variation for maintaining a conversion constant value for a 5.0% (w/v) lactose solution.

5. Conclusions

A mathematical model for an isothermal packed-bed immobilized enzyme reactor has been developed considering Michaelis–Menten kinetics with competitive product inhibition. Adding effects of intraparticle diffusion, external mass transfer, axial dispersion, biocatalyst swelling and deactivation effects predicted the experimental behaviour in the concentration range with errors lower than 2.5%. The influence of different parameters in the performance of packed bed immobilized enzyme reactor shows these behaviours:

(i) A non-linear dependence on axial reactor length of the progressively decreases steady state bulk substrate concentration, obtaining the higher conversion values in the reactor inlet for the smaller flow rates.
(ii) The swelling effect on the biocatalyst increases the superficial velocity of fluid into reactor, the biocatalyst deactivation by protein lost and the effective diffusion coefficient of lactose into biocatalyst, as the operation time is increased.
(iii) The influence of mass-transfer backmixing effects on concentration profile is less pronounced with the increase of operation time.
(iv) The positive influence of the swelling effect on the diffusion does not balance the negative influence that causes on the parameters of flow, added to the effect characteristic of the biocatalyst deactivation, and the conversion obtained for different feeding flows decays with the operation time.

These results are very important for reactor scaling up, without fitting previously the parameters at the working condition.

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Appendix A. Nomenclature

Species

E	β-galactosidase enzyme
EGa	enzyme–product complex (β-galactosidase-galactose)
ES	enzyme–substrate complex (β-galactosidase-lactose)
Ga	galactose
Gl	glucose
S	substrate (lactose)

Variables

a	specific area of the spherical catalyst particle (cm^{-1})

A_{kj}, B_{kj} coefficients of matrix A and B at the collocation point
C dimensionless substrate concentration inside catalyst spherical particle, $[S]/[S]_0^b$
C^* Dimensionless substrate concentration in the liquid stream, $[S]^b/[S]_0^b$
D biocatalyst particle diameter (cm)
D_r radial dispersion coefficient ($cm^2\ s^{-1}$)
D_S effective substrate diffusion coefficient (lactose) ($cm^2\ s^{-1}$)
D_z axial dispersion coefficient ($cm^2\ s^{-1}$)
f_i experimental factor according to proposed by Mackie and Meares
k_2 intrinsic constant for Michelis–Menten kinetic equation (mmol l^{-1} min^{-1} mg^{-1} protein)
k_d deactivation rate constant (h^{-1})
k_i intrinsic inhibition constant by product (mmol l^{-1})
k_l external mass transfer coefficient (cm s^{-1})
Km intrinsic Michaelis-Menten constant (mmol l^{-1})
n number of internal collocation points
Pe Peclet number, u_zZ/D_z
q_i coefficient of the polynomial equation
r radial coordinate (cm)
R biocatalyst particle radium (cm)
Re_p Reynolds number based on the particle, $D\ u_z\delta_L/\mu_L$
Sc Schmidt number, $\mu_L/(D_S\delta_L)$
Se_j Series of known functions to obtain an approximate solution of variables C^* and C in the x (0,1) and y (0,1) domains
Sh Sherwood number, k_lR/D_S
t operation time (h)
u_z superficial fluid velocity in the reactor (cm s^{-1})
V_{max} intrinsic maximum reaction rate (mmol l^{-1} min^{-1})
W weighting function for the applied method of orthogonal collocation
x dimensionless axial coordinate, z/Z
X substrate conversion
y dimensionless radial coordinate, r/R
z axial coordinate (cm)
Z reactor length (cm)

Greek letters

δ_L liquid stream viscosity (g cm^{-1} s^{-1})
ε void fraction of packed-bed reactor
γ dimensionless variable, $[S]_0^b/\mathrm{Km}$
γ_2 dimensionless variable, $([S]_0^b - [S])/k_i$
γ_2' dimensionless variable, $[S]_0^b/k_i$
μ_L liquid stream density (g cm^{-3})
ν reaction rate term
φ dimensionless variable, u_zR^2/D_SZ
φ_2 dimensionless variable, $u_zR^2[S]_0^b/(PeD_SZ)$
σ^2 variance of the concentration
ψ dimensionless variable, $8.483 \times 10^{-2}/(1 + Re_p)$
Φ^2 dimensionless variable, $V_{max}R^2/(\mathrm{Km}D_S)$

Symbols

[ ] concentration (mol l^{-1})

Subindexes

0 at initial time
aq in water

Subscripts

b in the liquid stream
i immobilized

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