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Kinetic model for lactose hidrolysis in a recirculation hollow-fibre bioreactor

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Abstract

Enzymatic hydrolysis of lactose by β-galactosidase (Lactozym) has been studied in a jacketed stirred-tank reactor (JSTR) and in a recirculation hollow-fibre bioreactor (RHFB). The hollow-fibre module was manufactured with modified cellulose-acetate membrane (Cuprophan). The variables tested were: temperature (25–40°C), lactose concentration (0.0278–0.208 M) and enzyme concentration (0.100–4.51 g l^{-1}). Within the experimental range analysed, the conversions in the RHFB, were lower than in the JSTR. A kinetic model is proposed for enzymatic hydrolysis of lactose in the RHFB, which considers competitive inhibition by the product (galactose) and reversible adsorption of the enzyme–galactose complex on the hollow-fibre membrane. The adsorption enthalpy of the enzyme–galactose complex onto the membrane was determined as being 77 5 kJ mol^{-1}.

Keywords: Enzyme; Kinetics; Membranes; Reaction engineering; β-Galactosidase; Lactose hydrolysis; Hollow-fibre bioreactor; Protein adsorption

1. Introduction

Enzymatic hydrolysis of lactose is an important biotechnological process because the delactosed products can be consumed by lactose maldigesters (Johnson et al., 1993), due to the formation of galacto-oligosaccharides that favour the growth of intestinal bacterial microflora (Shin et al., 1998) and to the improvement of the technological and sensorial characteristics of products containing hydrolysed lactose (Novo Nordisk, 1990).

The mechanism of enzymatic hydrolysis of lactose by β-galactosidase applied to different substrates (lactose solutions, whey and skim-milk) under different experimental conditions has been studied by several authors. Table 1 presents the kinetic models proposed by different researchers showing that most appropriate is a Michaelis–Menten kinetic model with competitive inhibition by galactose.

Lactose hydrolysis has been studied both with free enzyme (Santos et al., 1998) and immobilized in a packed-bed reactor (Illanes et al., 1998) or in membrane reactors (Bakken et al., 1992). Performance of β-galactosidase in hollow-fibre reactors has been studied previously (Kohlwey and Cheryan, 1981; Kim and Chang, 1983; Huffman and Harper, 1985;), these devices rendering lower conversion when working with enzyme in the free state.

Different works have demonstrated that protein–membrane interactions could trigger changes in the structure of the adsorbed molecules (Cheryan, 1998) and the protein could appear more open or denatured (Sheldon et al., 1991). This influences the activity of carbohydrase enzymes, such as α-galactosidase (Korus and Olson, 1977a), β-galactosidase (Kohlwey and Cheryan, 1981) and glucose isomerase (Korus and Olson, 1977b), seriously affected by polysulphone membranes.

There are many variables involved in protein–membrane interactions. The results in the literature regarding several aspects of proteins are sometimes contradictory and confusing. In general, the adsorption of proteins onto membranes depends on factors such as pH, ionic strength, isoelectric point of the protein, surface and protein properties (Norde et al., 1986), as well as the history dependence of protein-adsorption kinetics (Calonder et al., 2001). The affinity between a protein and an adsorbent generally increases with the hydrophobicity of the surface, and proteins desorb more easily from hydrophilic than from hydrophobic surfaces (Jönsson et al., 1987). In many instances,

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Table 1
Kinetic models proposed by different authors for enzymatic hydrolysis of lactose

Kinetic model proposed	References
Michaelis–Menten first order	Santos et al. (1996)
Michaelis–Menten without inhibition by product (galactose) (with enzyme–lactose complex formation)	Berrueta-Jiménez and García-Valle (1988)
Michaelis–Menten with competitive inhibition by product (total galactose)	Carrara and Rubiolo (1996), Illanes et al. (1998, 2000, 2001), Ladero et al. (2000), Papayannakos et al. (1993) and Santos et al. (1998)
Michaelis–Menten with competitive inhibition by product (total galactose) (considering $K_M = K_I$)	Jurado et al. (2002)
Michaelis–Menten with competitive inhibition by product (α- and β-galactose)	Flaschel et al. (1982), Peterson et al. (1989)
Michaelis–Menten with competitive inhibition by product (glucose)	Cavaille and Combes (1995)
Di-, tri- and tetrasaccharides formation	Zhou et al. (2003)

changes in the structure of a protein upon adsorption induce a certain irreversibility with respect to desorption (Norde, 1986; Norde et al., 1986). These structural alterations of the species adsorbed can bring about lower or a complete loss of activity. Several models of protein adsorption include a transition from a reversibly adsorbed state to a more tightly held state (Baszkin and Norde, 2000; Noinville et al., 2002).

The protein–membrane interactions in hollow-fibre-membrane bioreactors have also been investigated from a theoretical standpoint (Kim and Cooney, 1976; Davis and Watson, 1986; Rusu, 1998; Salzman et al., 1999; Calabró et al., 2002).

In the present work, enzymatic hydrolysis of lactose by β-galactosidase is studied in a recirculation hollow-fibre bioreactor (RHFB) manufactured with cellulose-acetate fibres (Cuprophan). We propose a kinetic model which considers competitive inhibition by the product and a reversible adsorption of the galactose–enzyme complex onto the membrane. The results in the RHFB are compared with those of a Jacketed stirred-tank reactor (JSTR).

2. Materials and methods

The chemical products used (PRS quality) were glucose, citric acid, K_2HPO_4, KCl, trichloroacetic acid (supplied by Panreac), $MgCl_2 \cdot 6H_2O$ (Prolabo), monohydrate lactose (Scharlau) and galactose (Across).

The enzyme used was a commercial β-galactosidase supplied by Novo Nordisk derived from a selected strain of the yeast *Kluyveromyces fragilis*, Lactozym 3000L HP-G [EC.3.2.1.23], which has a protein content of 35 g l^{-1}, $\rho = 1.2$ g ml^{-1}, with a declared activity of 3000 LAU ml^{-1} (1 LAU = commercial enzyme which can reach 1 μmol of glucose min^{-1} under standard conditions: 4.7% lactose concentration, pH = 6.5, 30°C, 30 min, standard milky buffer). This enzyme satisfies the specifications recommended for food enzymes.

The glucose was analysed applying the GOD-Perid method proposed by Werner et al. (1970) using a commercial reagent (Böehringer Mannheim Gmbh). The galactose and lactose present in the medium had no influence on the glucose determination. As the reaction medium, lactose solutions were prepared on a buffer 0.01 M K_2HPO_4, 0.015 M KCl and 0.012 M $MgCl_2 \cdot 6H_2O$ with pH = 6.75 adjusted with citric acid.

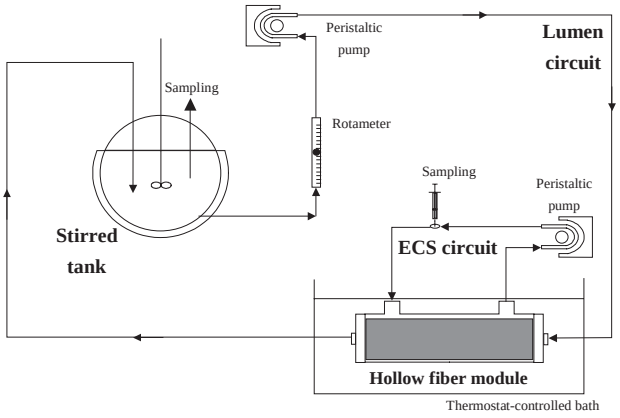

Fig. 1. Schematic diagram of the RHFB.

2.1. Experimental setup

Two reactors were used. In the JSTR, the enzymatic reaction took place in a 200 ml jacketed-stirred tank with pH and temperature controls. The kinetics of the hydrolysis were monitored by extracting samples from the reaction medium for a maximum period of 2 h, denaturing the enzyme with 0.1 N trichloroacetic acid, and measuring the glucose concentration according to reaction time by the GOD-Perid method.

Fig. 1 shows a schematic diagram of the RHFB. The setup consists of a jacketed-stirred tank (250 cm^3), two peristaltic pumps, a rotameter and a hollow-fibre module called NT1975 (membranes made in Cuprophan (cellulose acetate), surface area 1.95 m^2, size pore 5 kDa, supplied by Sorin Biomedica) submerged in a thermostat-controlled

Table 2
Variables tested for the lactose hydrolysis experiments in a RHFB

T (°C)	L_0 (M)	m (g)	C_{EC0} (g l^{-1})
25	0.0278	0.1250	0.752
	0.0694	0.0166, 0.0625, 0.1250, 0.3750	0.100, 0.376, 0.752, 2.26
	0.139	0.1250	0.752
	0.208	0.1250	0.752
30	0.0278	0.1250	0.752
	0.0694	0.0625, 0.1250, 0.1662, 0.3750, 0.7500	0.376, 0.752, 1.00, 2.26, 4.51
	0.139	0.1250	0.752
	0.208	0.1250	0.752
40	0.0278	0.1250	0.752
	0.0694	0.0625, 0.1250, 0.3750	0.376, 0.752, 2.26
	0.139	0.1250	0.752
	0.208	0.1250	0.752

bath. The module has two compartments, one formed by the fibre lumens (called lumen circuit) and the other being the space between fibres (called extracapillary space or ECS), where the enzyme is confined. The pore size permits the passage of substrate and reaction products but not the enzyme. Initially, the system was filled with 600 cm^3 of lactose solution and, when the working conditions were reached, the enzyme was added to the ECS circuit. One peristaltic pump maintained a constant circulation of 40 l h^{-1} in the lumen circuit while a second pump maintained a constant circulation of 53 l h^{-1} in the ECS circuit, functioning counter-current. The lactose-hydrolysis kinetics were monitored by extracting samples from the jacketed-stirred tank for a maximum period of 3 h, measuring the glucose concentration, according to reaction time by the GOD-Perid method. The variables tested are shown in Table 2. The experimental system was cleaned with a 1% solution of formaldehyde, flushing the system with distilled water before and after each experiment. After each hydrolysis experiment, it was confirmed that no enzyme had passed through the membrane.

2.2. Enzymatic activity

The enzymatic activity was measured in the following way: 1 ml of 50 g l^{-1} monohydrate lactose solution was added to 1 ml of 10 g l^{-1} enzyme solution, this being incubated at 30°C for 10 min. The reaction was stopped by mixing with 0.1 N trichloroacetic acid, measuring the glucose concentration by the GOD-Perid method. The enzymatic activity remained constant for the entire period of use (0.078 $mol_{glucose}\ g_{enzyme}^{-1}\ h^{-1}$).

To evaluate the thermal stability of the enzyme in the JSTR, we conducted experiments with pH and temperature controls. At regular intervals, for a maximum of 3 h, 1 ml samples were taken to measure the enzymatic activity. In the RHFB, the samples were extracted from the ECS circuit. The thermal-deactivation experiments were repeated three times, taking the mean value as the most probable. The error of the data was less than 5%.

3. Results and discussion

3.1. β-Galactosidase activity in the RHFB

The thermal deactivation of the enzyme in the RHFB was studied at 30°C, 40°C and 45°C, defining the normalized activity (a_{norm}) as the activity measured as a function of time divided by the initial activity. Fig. 2 shows the results for both reactors. The enzyme maintained practically all its activity after 3 h of analysis in the JSTR at 30°C (Vicaria, 2002). The same experiments performed in the RHFB, where the enzyme was retained in the ECS circuit, presented less activity. This could be due to an adsorption of the enzyme onto the membrane (Piret and Cooney, 1990; Taylor et al., 1994; Patkar et al., 1995), to a denaturing of this caused by conformational changes on making contact with the membrane of the hollow-fibre module (Kohlwey and Cheryan, 1981; Cheryan, 1998), or else due to pumping (Huffman and Harper, 1985). The loss of activity caused by the alteration of the structural conformation of the proteins, as a consequence of shearing forces, has been indicated by different authors (Volkin and Klibanov, 1989).

In an effort to determine whether pumping was significant under the working conditions tested, the enzymatic hydrolysis of lactose was performed in a JSTR (initial conditions: $C_{E0}=0.50$ g l^{-1}, $L_0=0.0278$ M, 40°C). Instead of magnetic stirring, we recirculated the solution by a peristaltic pump that worked under the same conditions as the pump of the ECS circuit. The results in both cases were similar (Vicaria, 2002), ruling out the idea that shearing forces caused by the peristaltic pump deactivated the enzyme used. This lesser activity detected in the RHFB, in the absence of lactose,

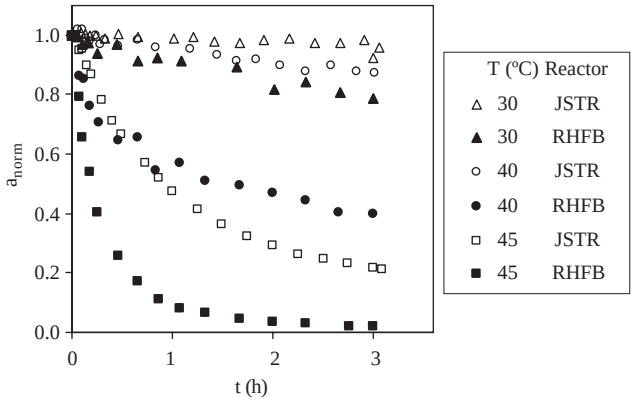

Fig. 2. Comparison of enzymatic activity of β-galactosidase in the JSTR and RHFB.

may possibly be due to an adsorption or accumulation of the free enzyme onto the membrane.

3.2. Model for the RHFB

In the RHFB, when the perfect-mix hypothesis is applied to the jacketed stirred tank as well as the ECS circuit of the bioreactor (given the intense stirring of both), a balance of glucose to the system leads to

$$V\frac{\mathrm{d}G}{\mathrm{d}t} = V_c C_{\mathrm{EC}} r_{\mathrm{HF}} \tag{1}$$

with V being the volume of the liquid phase in RHFB, G the glucose concentration, V_c the volume of the liquid phase in the ECS circuit, C_{EC} the enzyme concentration in the ECS circuit and r_{HF} the reaction rate in the RHFB. Introducing the enzyme mass (m) and the conversion (x), we get the equation:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{m r_{\mathrm{HF}}}{V L_0}. \tag{2}$$

As a means of making the treatment more general, the intensive-treatment variable (y) used was defined as

$$\mathrm{d}y = C_E\,\mathrm{d}t = \frac{m}{V}\,\mathrm{d}t \Rightarrow y = \sum m_i \frac{\Delta t_i}{V_i}, \tag{3}$$

where C_E is the total active enzyme concentration, Δt_i the time between the consecutive samplings, m_i the enzyme mass in the bioreactor and V_i the volume of the liquid phase. In this way, the variation in the total volume of the liquid phase can be taken into account in addition to the enzyme removed during the samplings, in both experimental systems.

Taking into account Eq. (3) in Eq. (2), we find that

$$r_{\mathrm{HF}} = L_0 \frac{\mathrm{d}x}{\mathrm{d}y}. \tag{4}$$

The expression determining the reaction rate in the RHFB coincides with that for the JSTR (Eq. (12)).

3.3. Kinetic model of enzymatic hydrolysis with competitive inhibition by galactose

In a previous work, Jurado et al. (2002) explained the enzymatic hydrolysis performed in a JSTR, proposing a kinetic model that considered the competitive inhibition of the enzyme by the product (galactose):

$$E + L \overset{K_M}{\rightleftarrows} EL, \tag{5}$$

$$EL \overset{k}{\rightarrow} EGa + G, \tag{6}$$

$$EGa \overset{K_I}{\rightleftarrows} E + Ga, \tag{7}$$

where E is the concentration of free enzyme present in the reaction medium, K_M and K_I are the equilibrium constants of the reactions indicated in Eqs. (5) and (7), and k the reaction constant of the limiting step.

The lactose (L), galactose (Ga) and glucose (G) concentrations were defined as a function of the conversion:

$$L = L_0(1-x), \tag{8}$$

$$Ga = Ga_0 + L_0 x, \tag{9}$$

$$G = G_0 + L_0 x, \tag{10}$$

$$S_0 = L_0 + Ga_0. \tag{11}$$

From the definition of S_0 formulated, under the assumption that $K_M = K_I$ (implying that the enzyme has almost the same affinity for the substrate (lactose) as for the reaction product (galactose) (Jurado et al., 2002), the reaction rate in the JSTR (r) would be defined as

$$r = L_0 \frac{\mathrm{d}x}{\mathrm{d}y} = \frac{ke_T L_0(1-x)}{K_M + S_0}, \tag{12}$$

where e_T represents the mols of active enzyme per gram of the active enzyme used. By the integration and application of the simplification proposed, the intensive treatment variable (y) is defined by

$$y = \left(\frac{K_M}{ke_T} + \frac{1}{ke_T} S_0\right)[-\ln(1-x)]. \tag{13}$$

The constants K_M and ke_T can be determined as a function of temperature using the expressions (Jurado et al., 2002):

$$K_M = 2.77 \times 10^2 \exp\left(\frac{-3210}{T}\right),$$

$$ke_T = 2.71 \times 10^7 \exp\left(\frac{-5630}{T}\right).$$

3.4. Kinetic model for lactose hydrolysis in an RHFB

Applying the kinetic model defined by Eq. (13) to the experiments made in the RHFB (Figs. 3–5), we found that it adjusted only during a certain reaction interval, after which the experiments deviated from the model proposed and the

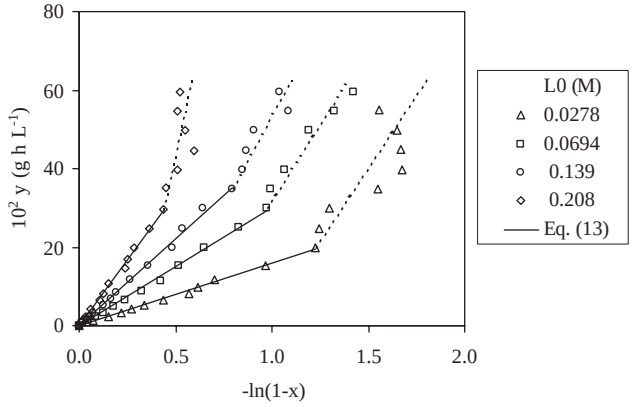

Fig. 3. Influence of initial lactose concentration on lactose hydrolysis in the RHFB. Fit of the experimental results at 40°C, $m = 0.1250$ g to Eq. (13).

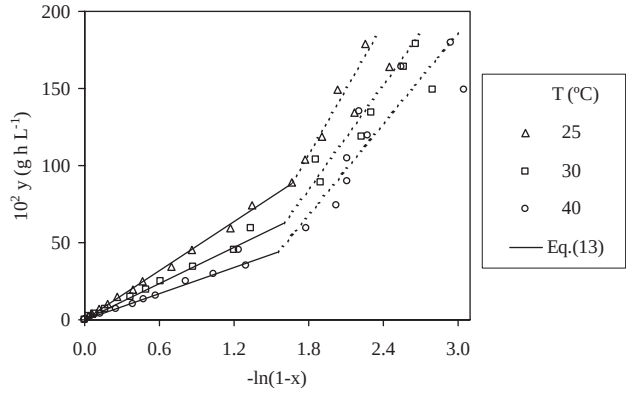

Fig. 4. Influence of temperature on lactose hydrolysis in the RHFB. Fit of the experimental results at $L_0 = 0.0694$ M, $m = 0.3750$ g to Eq. (13).

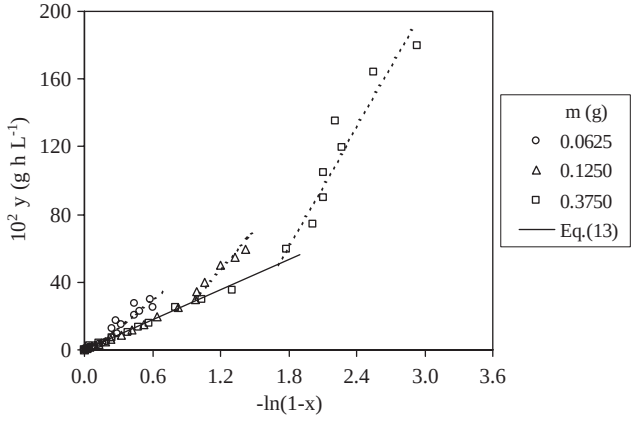

Fig. 5. Influence of enzyme concentration on lactose hydrolysis in the RFHB. Fit of the experimental results at 40°C, $L_0 = 0.0694$ M to Eq. (13).

variation of the conversion became barely significant with the passage of the reaction time. This behavioural change appears to occur at lower conversion values, the higher the substrate concentration and reaction temperature (Figs. 3 and 4) and at greater conversion values when the enzyme concentration is increased (Fig. 5).

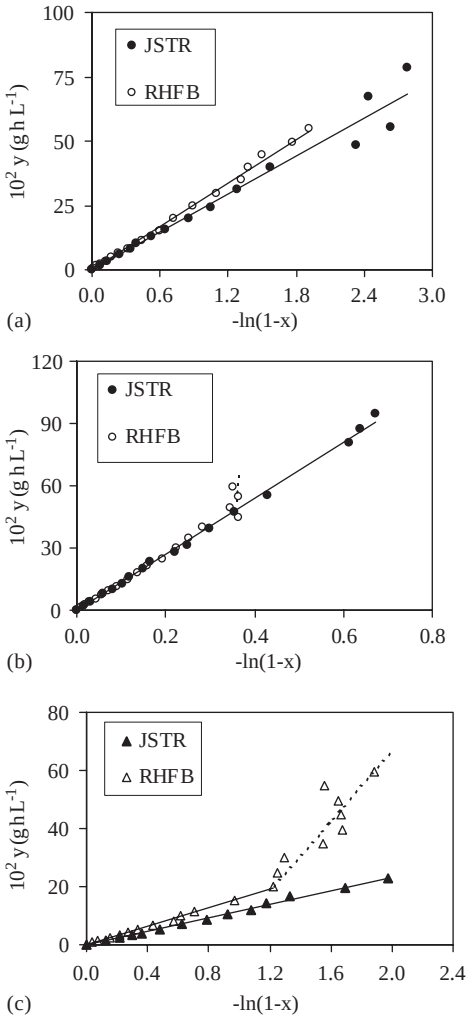

Fig. 6. Lactose hydrolysis in the JSTR and RHFB. Variation of y vs. $[-\ln(1-x)]$ (Eq. (13)): (a) 25°C, L_0=0.0278 M, (b) 25°C, L_0=0.208 M, and (c) 40°C, $L_0 = 0.0278$ M.

Also, the conversion found for the RHFB was less than for the JSTR in all the experiments made with both systems operating under similar experimental conditions (Fig. 6). These figures show that the differences of behaviour between the two reactors augment at higher temperatures (Figs. 6a and c) and lower substrate concentrations (Figs. 6b and c), becoming least notable at 25°C and at an initial substrate concentration of 0.208 M (Fig. 6b). The lower activity of the enzyme in the RHFB could be due to an adsorption or accumulation of the enzyme onto the membrane, and the deviation from the model after a time, could be caused by some reaction product. The enzyme did not deactivate due to the shearing forces caused by the ECS-circuit pumping. Experiments on glucose and lactose circulation through the RHFB membrane also showed that resistance to the transfer of these sugars through the membrane and the liquid

films was negligible. Lactose-hydrolysis experiments made at different temperatures in the JTSR reactor demonstrated that in the presence of lactose, the enzyme did not deactivate (Jurado et al., 2002, 2003). In addition, we performed experiments of lactose hydrolysis at 40°C using Lactozym, with a substrate concentration (25 g l^{-1}) in which different initial enzyme concentrations (0.1–3.0 g l^{-1}) were assayed to test the influence that the presence of lactose has on the enzymatic deactivation. The x vs. e_0t representation shows that the experimental results are fitted to the same line, and therefore it appears that lactose stabilizes the enzyme, according to the postulate by Mahoney et al. (1988), Yang et al. (1994) and Illanes et al. (1998).

The adsorption of proteins onto membranes has been amply demonstrated in the literature (Wojciechowski and Brash, 1990; Gekas et al., 1993; Cheryan, 1998), some works proposing kinetic models that include the adsorption of the free enzyme onto the membrane to explain fructose–glucose isomerization in the RHFB (Jurado et al., 2000) or adsorption isotherms following Freundlich laws (Aimar et al., 1986).

Initially, for the interpretation of the hydrolysis experiments, kinetic models that took into account the adsorption of the free enzyme E and the EL complex on the membrane were proposed. However, these models did not satisfactorily fit the experimental results. From the foregoing observations, we propose a model which considers that the species adsorbed is fundamentally the enzyme–galactose complex (EGa), the concentration of which increases as the reaction progresses. The kinetic mechanism would be equal to that proposed previously (Eqs. (5)–(7)), adding a step that considers the adsorption of the enzyme on the free active centres of the membrane:

$$EGa + n \overset{K_A}{\rightleftarrows} EGan, \tag{14}$$

where n is the concentration of the free active centres on the membrane of the hollow-fibre module and $EGan$ the concentration of the enzyme–galactose complex adsorbed onto the active centres of the membrane. The equilibrium constant K_A is defined as

$$K_A = \frac{EGan}{EGa\, n}. \tag{15}$$

In consideration that the membrane surface is very large with respect to the enzyme concentration assayed, the concentration of empty active centres for the adsorption on the membrane surface (n) can be assumed constant and can be included in the adsorption constant (K_D) in the form

$$K_D = K_A\, n. \tag{16}$$

Given that $K_M \approx K_I$ (Jurado et al., 2002), the concentration of the enzyme–galactose complex adsorbed onto the membrane ($EGan$) would be equal to

$$EGan = E\,\frac{K_D}{K_M}\,(Ga_0 + L_0x). \tag{17}$$

Applying the estimate of the stationary state to the EL, EGa and $EGan$ complex, the balance to the free enzyme, E, would be defined as

$$E = \frac{e_T C_E}{1 + (S_0/K_M) + (K_D/K_M)\,(Ga_0 + L_0x)}. \tag{18}$$

The appearance of the conversion of the denominator of this equation indicates a continuous decline in free-enzyme concentration with the progression of the reaction. The rate of the lactose-hydrolysis reaction in the RHFB is determined by the controlling step (Eq. (6)), and therefore r_{HF} is defined as

$$r_{HF} = L_0\,\frac{\mathrm{d}x}{\mathrm{d}y} = \frac{ke_T L_0(1-x)}{K_M + S_0 + K_D(Ga_0 + L_0x)}. \tag{19}$$

Integration gives

$$y = \frac{K_M + S_0(1+K_D)}{ke_T}\,[-\ln(1-x)] - \frac{K_D L_0}{ke_T}\,x. \tag{20}$$

An application of Eq. (20) to the experimental results for the RHFB and the values of ke_T and K_M in the JSTR enabled, by non-linear regression, the determination of the value of the K_D constant (Table 3). The K_D constants calculated at different temperatures indicated that adsorption increased with temperature, as Cheryan (1998) indicated for the protein adsorption onto different membranes. Fig. 7 shows the fit of the kinetic constants K_D to the Van't Hoff equation, giving an adsorption enthalpy (ΔH_{ad}) of 77.5 kJ mol^{-1}, the order of magnitude of which reveals a chemical adsorption or chemisorption of the enzyme–galactose complex onto the membrane. Similar values

Table 3
Adsorption constant K_D calculated by non-linear regression (Eq. (20))

T (°C)	K_D	CV (%)
25	0.667	5
30	1.09	3
40	2.97	3

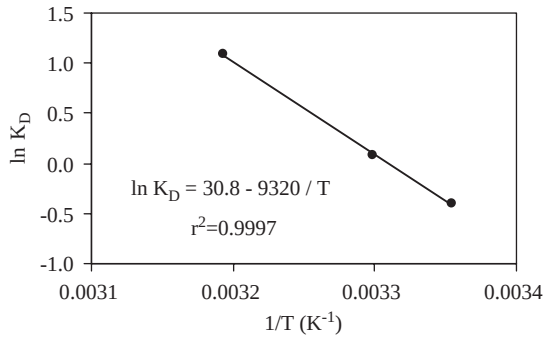

Fig. 7. Lactose hydrolysis in the RHFB. Fit of K_D to the Van't Hoff equation.

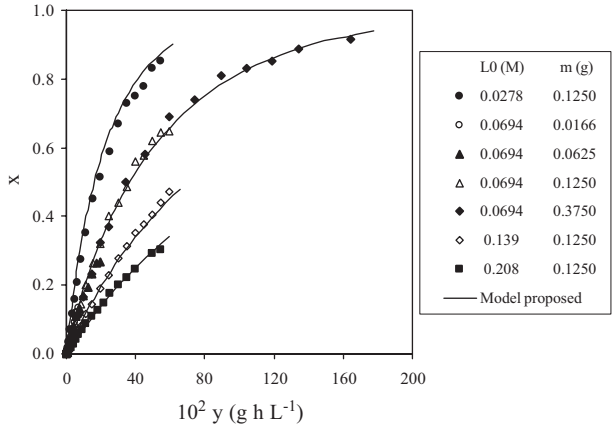

Fig. 8. Lactose hydrolysis in the RHFB. Model of adsorption of the enzyme–galactose complex onto the membrane at 25°C.

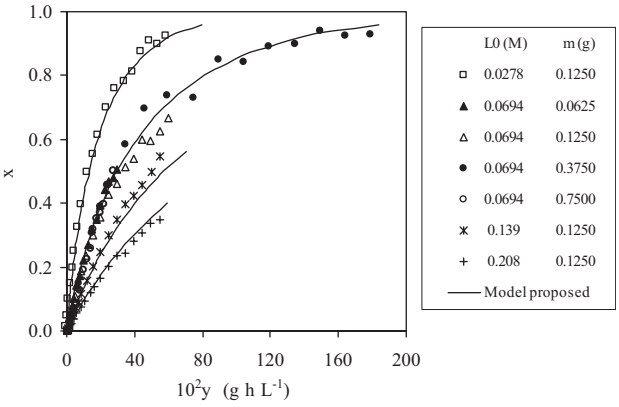

Fig. 9. Lactose hydrolysis in the RHFB. Model of adsorption of the enzyme–galactose complex onto the membrane at 30°C.

have been reported previously by Norde et al. (1986) who calorimetrically measured the adsorption enthalpy of human-plasma albumin (HPA) on α-Fe_2O_3 (200 kJ mol^{-1}) and on silica (136 kJ mol^{-1}). Both in the experiments performed in the present work, the adsorption occurred spontaneously and therefore $\Delta G_{ad} = \Delta H_{ad} - T \cdot \Delta S_{ad} < 0$. As this reaction is endothermic ($\Delta H_{ad} > 0$) implies that the adsorption is driven by an increase in entropy ($T \cdot \Delta S_{ad} > 0$), as reported in Lee and Ruckenstein (1988) and Norde et al. (1986).

Figs. 8 and 9 show the comparison between the experimental results in the RHFB and the predictions by the model for some of the temperatures assayed. The residuals calculated were distributed randomly to one side or the other of the adjustment curve, with more than 55% of the experimental points differing by less than 5% with respect to those calculated from the model. The model proposed enables the interpretation of the interaction between the enzyme β-galactosidase and the RHFB membrane. From Eqs. (17) and (18) and constants K_M and K_I as a function of temperature, we can simulate the variations in the concentrations of the different enzymatic species as a function of the conversion. Fig. 10 shows the distribution of the different enzymatic species at 40°C in the RHFB for an initial lactose concentration of 0.208 M. At almost all conversion values, the free-enzyme concentration was far lower than the rest of the enzyme complexes formed. Similarly, the enzyme complex adsorbed onto the membrane (*EGan*) was roughly 50% of the total enzyme for a conversion of 0.40, where the deviation from the data began with respect to Eq. (13). An analysis of the influence that temperature and the initial substrate concentration exert on the quantity of enzyme–galactose complex adsorbed onto the membrane shows (Fig. 11) that, for the same conversion, the concentration of the *EGan* complex increases with a greater initial substrate concentration and higher temperature.

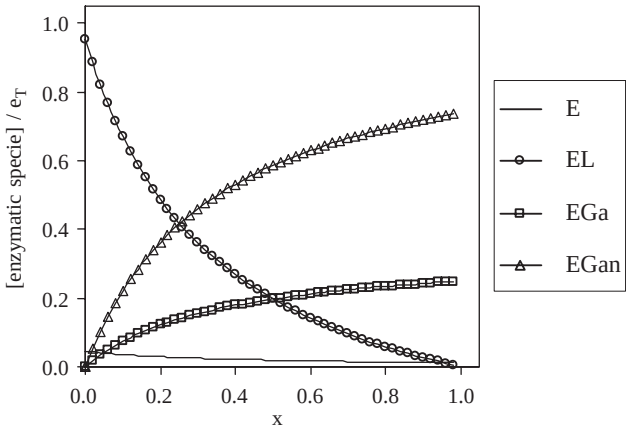

Fig. 10. Simulation: different enzymatic species present in the lactose hydrolysis in the RHFB at 40°C, $L_0 = 0.208$ M, $Ga_0 = 0$ M.

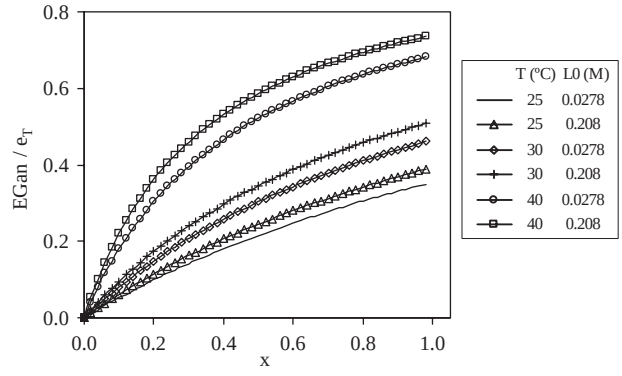

Fig. 11. Simulation: influence of the temperature and initial substrate concentration on the *EGan* adsorbed onto the membrane.

4. Conclusions

The enzymatic activity in the RHFB was lower than in a JSTR possibly due to the removal of the enzyme by adsorption onto the membrane and/or structural changes in the enzyme during the adsorption process.

The conversion detected in the RHFB was lower than in the JSTR. This lower conversion was due to a reversible adsorption of the enzyme–galactose complex onto the membrane and occurred during the reaction process. A kinetic model has been proposed for the enzymatic hydrolysis of lactose in an RHFB, which considers competitive inhibition of the enzyme by galactose, in which the constant $K_M = K_I$ and which includes the reversible adsorption of the enzyme–galactose complex onto the RHFB membrane. The model proposed provides a satisfactory fit of the experimental results in the RHFB. The adsorption constant of the enzyme–galactose complex onto the membrane can be determined at different temperatures:

$$K_D = 2.38 \times 10^{13} \exp\left(\frac{-9320}{T}\right), \quad (21)$$

giving a value of 77.5 kJ mol^{-1} for the adsorption enthalpy.

Notation

a_{norm}	normalized activity (dimensionless)
C_E	concentration of total active enzyme, g l^{-1}
C_{EC}	concentration of total active enzyme in ECS circuit, g l^{-1}
CV	covariance, %
ΔG_{ad}	free-energy increase during adsorption
ΔH_{ad}	adsorption enthalpy, kJ mol^{-1}
ΔS_{ad}	entropy increase during adsorption
e_T	mols of active enzyme per gram of active enzyme, mol g^{-1}
E	concentration of free enzyme present in the reaction medium, M
ECS	extracapillary space
EGa	concentration of the enzyme–galactose complex, M
EGan	concentration of the enzyme–galactose complex adsorbed onto the active centres of the membrane, M
EL	concentration of the enzyme–lactose complex, M
G	concentration of glucose, M
Ga	concentration of galactose, M
GOD	glucose oxidase
JSTR	jacketed stirred-tank reactor
k	rate constant of Eq. (6), ($mol_{glucose}\ mol^{-1}_{enzyme}\ h^{-1}$)
K_A	equilibrium constant of Eq. (14), M^{-1}
K_D	adsorption constant (dimensionless)
K_I	equilibrium constant of Eq. (7), M
K_M	Michaelis–Menten constant, M
L	concentration of monohydrate lactose, M
m	enzyme mass, g
n	concentration of free active centres on the membrane of the hollow-fibre module, M
r	reaction rate in the JSTR, mol $g^{-1}\ h^{-1}$
r_{HF}	reaction rate in the RHFB, mol $g^{-1}\ h^{-1}$
RHFB	recirculation hollow-fibre bioreactor
S_0	sum of concentration of initial lactose and galactose, M
t	time, h
T	temperature
V	volume of liquid in the RHFB, l
V_c	volume of liquid in the ECS circuit, l
x	conversion (dimensionless)
y	intensive-treatment variable, g h l^{-1}

Subscript

0	initial concentrations

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