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Kinetic models of activity for β-galactosidases: influence of pH, ionic concentration and temperature

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Abstract

The influence that different experimental conditions have on the activity of two commercial β-galactosidases (Lactozym and Maxilact) has been investigated. Two kinetic models have been proposed to explain the behaviour of the enzymatic activity versus pH, implying the dissociation of one or two protons of the enzyme. The thermal deactivation found for the enzyme β-galactosidase was fit to a kinetic model. The kinetic parameters have been calculated.

The models proposed explain the behaviour of the enzyme with different pH and temperature values, and satisfactorily fit the experimental results obtained in this work as well as the results proposed by other researchers for lactases from different sources.

Keywords: β-Galactosidase; Deactivation models; pH; Ionic concentration; Temperature; Activity

1. Introduction

The study of the stability of enzymes is an important aspect to consider in biotechnological processes, as this can provide information on the structure of the enzymes and facilitate an economical design of continuous processes in bioreactors. Deactivation mechanisms can be complex, since the enzymes have highly defined structures, and the slightest deviation in their native form can affect their specific activity. Better knowledge of enzyme stability under operating conditions could help optimize the economic profitability of enzymatic processes.

The activity and thermal stability of enzymes is influenced by diverse environmental factors (temperature, pH, reaction medium, shaking, shearing) which can strongly affect the specific tridimensional structure or spatial conformation of the protein [1–5]. The combination of different factors that can simultaneously deactivate the enzyme complicate the interpretation of the activity data. These effects will be more completely understood when the tridimensional structures of the enzymes, and how these are influenced by the environment, are known.

Therefore, knowledge of the effects that different environmental factors has on enzymatic activity and molecular structure would be highly useful to industrial applications. One of the most studied factors affecting the activity and stability of β-galactosidase is the influence of such ions as Ca^{2+}, Mg^{2+}, Na^{+}, $NH_4{}^{+}$ and K^{+}. Regardless of the origin of the enzyme, in all the works consulted, it is indicated that Ca^{2+} ions inhibit the functioning of the enzyme [6], while Mg^{2+} ions increase their activity. These latter ions are essential to avoid the deactivation in certain cases [6]. On the contrary, the effect that $NH_4{}^{+}$, K^{+} and Na^{+} ions have on the enzymatic activity and stability varies according to the author and species analysed [2,7]. The effect caused by these ions appears to be related to the radii of the monovalent ions, so that the smallest ions, such as Na^{+} can enter the structure of the protein, inducing conformational changes in the enzyme structure which are able to deactivate the enzyme. Contrarily, the presence of $NH_4{}^{+}$ and K^{+}, which have a similar ionic radius, boost the activity of the enzyme. Irrespective of the influence that these ions can have on enzymatic activity, they can also strengthen the resistance of the protein to thermal inactivation by reducing the flexibility of the polypeptide backbone.

Among the most widely used models to explain the thermal deactivation of β-galactosidases is the first-order deactivation model:

$$E \xrightarrow{k_d} E_d \quad (1)$$

where E is the concentration of native enzyme in the reaction medium, E_d the concentration of deactivated enzyme and k_d the deactivation-kinetic constant. This model appears to

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Table 1
Deactivation models and activation energy (E_a) proposed for β-galactosidases by different authors

References	Enzyme source	Buffer	Deactivation model	T (°C)	E_a (kcal mol^{-1}) (sol.)	E_a (kcal mol^{-1}) (imm.)
[8]	*Kluyveromyces lactis* cells	Phosphate buffer	Eq. (1)			7.78
[9]	*Aspergillus niger*	Buffer with lactose	Eq. (1)	55–60	82.3	92.5
[10]	*Kluyveromyces fragilis*		Eq. (1)	43		
[11]	*Escherichia coli*		Eq. (1)	58–64	184 (in vivo) 108 (in vitro)	
[12]	*Bacillus circulans*		Eq. (1)	52–68		43.3
[13]	*Aspergillus niger*		Eq. (1)	25–60		55.7
[14]	*Kluyveromyces lactis*	Phosphate buffer with K^+ and Mg^{2+} ions	Eq. (1)	35–55		47.8
[15]	*Kluyveromyces lactis*	Phosphate buffer with K^+ and Mg^{2+} ions	Eq. (2)	45–53		
[16]	*Kluyveromyces fragilis*	Buffer with K^+ and Mg^{2+} ions	Eq. (14)[a]	40–50	77.8	
This work	*Kluyveromyces fragilis*/*Kluyveromyces lactis*	Buffer[b] K^+ and Mg^{2+}	Eq. (14)[a]	30–50	108	
This work	*Kluyveromyces fragilis*/*Kluyveromyces lactis*	Buffer[b] Na^+	Eq. (14)[a]	25–40	44.7	

sol.: soluble enzyme; imm.: immobilized enzyme.
[a] Model proposed in this work.
[b] Buffer defined in this work.

reproduce mainly the experimental results for immobilised enzymes (Table 1). The form of first-order deactivation kinetics may be attributed primarily to the disruption of a single bond or sensitive structure, or the occurrence of a single lethal event or single hit [17].

To explain enzymatic deactivation of the enzymes, a deactivation series model has been proposed by [5]:

$$E \xrightarrow{k_1} \underset{\alpha_1}{E_1} \xrightarrow{k_2} \underset{\alpha_2}{E_2} \quad (2)$$

This model considers two irreversible first-order steps and the presence of native enzyme (E) as well as modified enzymatic species (E_1, E_2), the latter two with a specific activity differing from that of the native enzyme. In the model, α_1 and α_2 are, respectively, the relative activities of E_1 and E_2 with respect to the specific activity of E. This model has been applied to experimental results involving β-galactosidases by [15], who determined k_1, k_2, α_1 and α_2 of immobilised lactases from *Escherichia coli* and *Kluyveromyces lactis* in different reaction mediums. The data are calculated for a single temperature, and therefore the fit of the kinetic constants to the Arrhenius equation cannot be verified.

In the previous model, if $k_2 = 0$:

$$E \xrightarrow{k_1} \underset{\alpha_1}{E_1} \quad (3)$$

This model was also used by [15] to explain the enzymatic deactivation of β-galactosidases.

Another model habitually accepted to explain the process of the denaturing of the proteins is the one proposed by [18] and applied by [1–3,17,19]. According to this model, protein can be transformed from an active native state (N) to a non-active state (D), this process being reversible. The enzyme D can also evolve to a non-active state (I), this being the irreversible step:

$$N \leftrightarrow D \rightarrow I \quad (4)$$

Nevertheless, most works analysed treat the stability of lactases under the experimental conditions used for lactose hydrolysis. Only a few authors include enzymatic deactivation within the kinetic model of lactose hydrolysis by β-galactosidases [9,10,13]. This may be because the enzyme is stabilised by the substrate (lactose) or the product (galactose), as is demonstrated by [19–21]. This implies that the enzymatic deactivation does not occur during the hydrolysis reaction.

Table 1 summarises the kinetic models proposed by different authors that have studied the thermal deactivation of β-galactosidases. The models proposed and activation energies calculated are shown.

In the present work, we analyse the activity of two β-galactosidase enzymes from different sources under contrasting experimental conditions. We study the influence of pH on the enzymatic activity and the thermal deactivation with different ionic concentrations proposing models that explain the results found. The good fit on applying the kinetic models proposed to the experimental data reported by other authors corroborates the assumptions considered.

2. Materials and methods

2.1. Enzymes and enzymatic activity

The enzymes used were two commercial β-galactosidases:

- *Lactozym 3000L HP-G* (EC 3.2.1.23), derived from a selected strain of the yeast *Kluyveromyces fragilis* (sup-

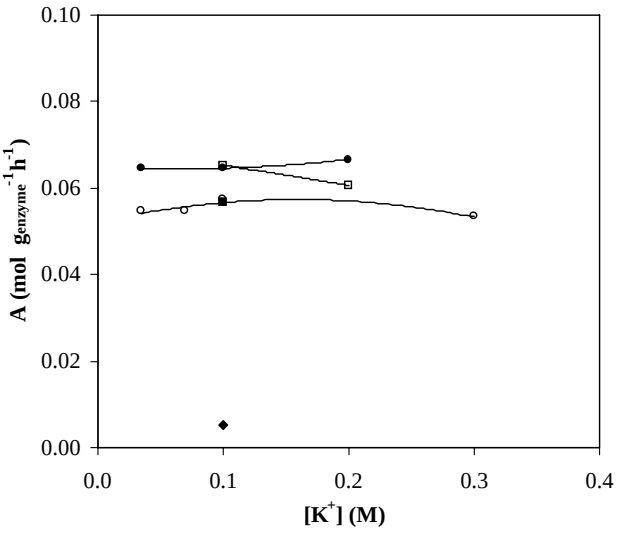

Fig. 1. Influence of pH and [K^+] on the activity of Lactozym. Citric-phosphate buffer, [PO_4^{3-}] = 0.01 M, fit of [K^+] by KCl addition, [Mg^{2+}] = 0 M. (◆) pH 6.00, (○) pH 6.50, (●) pH 6.75, (□) pH 7.00 and (■) pH 7.30.

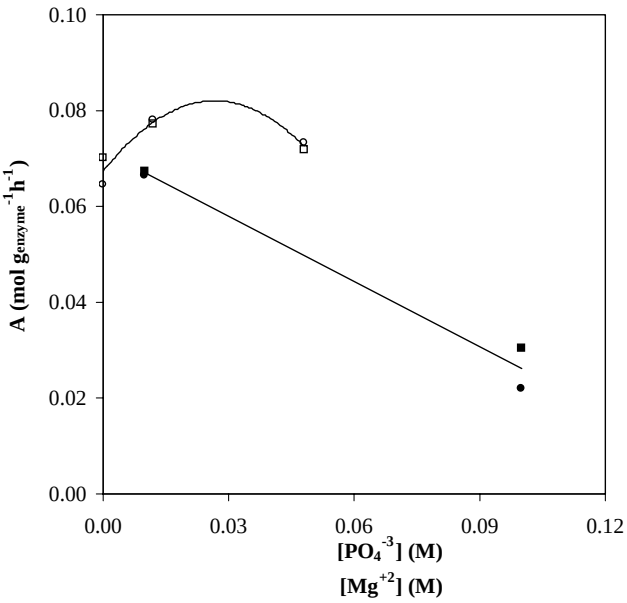

Fig. 2. Influence of [PO_4^{3-}] and [Mg^{2+}] on the activity of Lactozym and Maxilact. Citric-phosphate buffer: (a) influence of [PO_4^{3-}]: (●)Lactozym, (■) Maxilact (pH = 6.75, [K^+] = 0.2 M, [Mg^{2+}] = 0 M); (b) influence of [Mg^{2+}]: (○) Lactozym, (□) Maxilact (pH = 6.75, [PO_4^{3-}] = 0.01 M, [K^+] = 0.035 M).

plied by Novo Nordisk), has a protein content of 35 g l^{-1}, ρ = 1.2 g ml^{-1} and a declared activity of 3000 LAU ml^{-1} (1 LAU: commercial enzyme which can produce 1 μmol of glucose per minute under standard conditions: 4.7% lactose concentration, pH = 6.5, 30 °C, 30 min, standard milky buffer [22]).

- *Maxilact-L/2000* (EC 3.2.1.23), derived from a selected strain of the yeast *K. lactis* (supplied by Gist-Brocades), has a declared activity of 2000 NLU (1 NLU: mass of commercial enzyme which produces 1 μmol of ONP [*o*-nitrophenol] from a ONPG solution [*o*-nitrophenyl β-D-galactopyranoside] under standard conditions [23]).

The enzymatic activity was measured in test tubes at 30 °C as follows: 1 ml of 50 g l^{-1} monohydrate lactose solution prepared on the buffer selected was added to 1 ml of 10 g l^{-1} enzyme solution prepared on the same buffer. The test tube was incubated at 30 °C for 10 min, after which 1 ml was extracted. The reaction was stopped by mixing with 1 ml of 0.1N trichloroacetic acid. Afterwards, the glucose concentration was measured by the GOD-Perid method [24] using a commercial reagent (Böehringer Mannheim GmbH). The galactose and lactose present in the medium had no influence on the glucose determination.

2.2. Influence of different ions on enzymatic activity

To optimize the conditions under which Lactozym and Maxilact act, we analysed the behaviour of both at different pH values and ionic concentrations that maximize the enzymatic activity, as is indicated by [6,7,19]. The results, Figs. 1 and 2, show the influence that pH and K^+, PO_4^{3-} and Mg^{2+} ions have on the activity. The behaviour of Lactozym and Maxilact was similar. As an example, Fig. 1 shows the influence that pH and K^+ ions have on Lactozym activity, indicating that the K^+ concentration do not appreciably alter the enzymatic activity. Thus, Fig. 2 shows that there is an optimal concentration of Mg^{2+} ion for both enzymes and that the increase in the PO_4^{3-} concentration considerably diminished the enzymatic activity.

Within the experimental range studied, the highest activity was registered with the buffer called Buffer K^+ and Mg^{2+}, having a composition of 0.01 M in K_2HPO_4, 0.015 M in KCl and 0.012 M in $MgCl_2{\cdot}6H_2O$, fitted to pH = 6.75 by citric acid. The activity of both enzymes in this buffer remained constant over the entire experimental period. The activity values (*A*) were quite similar for both enzymes (78×10^{-3} and 77×10^{-3} $mol_{glucose}$ g_{enzyme}^{-1} h^{-1} for Lactozym and Maxilact, respectively).

In addition, the presence of Na^+ ions was analysed using a buffer called Buffer Na^+ (0.07 M in $Na_2HPO_4{\cdot}12H_2O$, citric acid, pH = 6.5) as a reaction medium. The Lactozym and Maxilact activity was similar, and throughout the experimental period remained constant and equal to 3.06×10^{-3} and 3.04×10^{-3} $mol_{glucose}$ g_{enzyme}^{-1} h^{-1}, respectively, values far lower than those registered with Buffer K^+ and Mg^{2+}.

2.3. Thermal stability of the enzymes

To evaluate the thermal stability of both enzymes, we performed experiments in a 250 ml stirred-tank batch reactor with pH and temperature controls. The enzymatic solution

was stirred at 700 rpm. At regular intervals, for a maximum of 3 h, 1 ml samples were taken to measure the enzymatic activity. 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. Influence of pH on enzymatic activity

The influence of pH on β-galactosidases activity is generally analysed only to determine the optimal pH range, without proposing kinetic models that might explain this dependence. The effect of pH on enzymatic activity is usually explained by a kinetic model in which the enzyme undergoes deprotonation, according to the model:

$$EH^+ \leftrightarrow E + H^+ \tag{5}$$

$$E \leftrightarrow E^- + H^+ \tag{6}$$

The equilibrium constants of the reactions, K_1 and K_2, were defined as:

$$K_1 = \frac{[E][H^+]}{[EH^+]}, \qquad K_2 = \frac{[E^-][H^+]}{[E]} \tag{7}$$

Making an overall balance for the enzyme, and considering that only the native enzyme (E) is active, we found that the activity A (mol g_{enzyme}^{-1} h^{-1}) under substrate-saturation conditions would vary with pH according to the expression:

$$A = \frac{V_{max}}{1 + 10^{pK_1 - pH} + 10^{pH - pK_2}} \tag{8}$$

Another model that may explain the behaviour of the enzymatic activity with pH is the one that considers the dissociation of two protons according to the following equations:

$$EH_2^{2+} \leftrightarrow E + 2H^+ \tag{9}$$

$$E \leftrightarrow E^- + H^+ \tag{10}$$

For this model, the equilibrium constants of the reactions, K_1 and K_2, are defined as:

$$K_1 = \frac{[E][H^+]^2}{[EH_2^{2+}]}, \qquad K_2 = \frac{[E^-][H^+]}{[E]} \tag{11}$$

Making an overall balance for the enzyme and considering that only the native enzyme is active, we found that the activity A (mol g_{enzyme}^{-1} h^{-1}) under substrate-saturation conditions varies with pH according to the expression:

$$A = \frac{V_{max}}{1 + 10^{pK_1 - 2pH} + 10^{pH - pK_2}} \tag{12}$$

The activity data versus pH was similar in Lactozym and Maxilact in a citric-phosphate buffer (ionic concentration similar to Buffer K^+ and Mg^{2+} with pH altered by citric acid addition). Both models were applied to the activity data determined experimentally and the fit by non-lineal regression enabled us to calculate the parameters shown in Table 2. Fig. 3 shows that the difference between the two proposed models is not very significant, although the r^2 value found on applying the models (Table 2) would indicate that the model which assumes the dissociation of two protons (Eqs. (9) and (10)) reproduces the experimental data better. Nevertheless, it would be useful to increase the number of experiments to test the conclusions drawn.

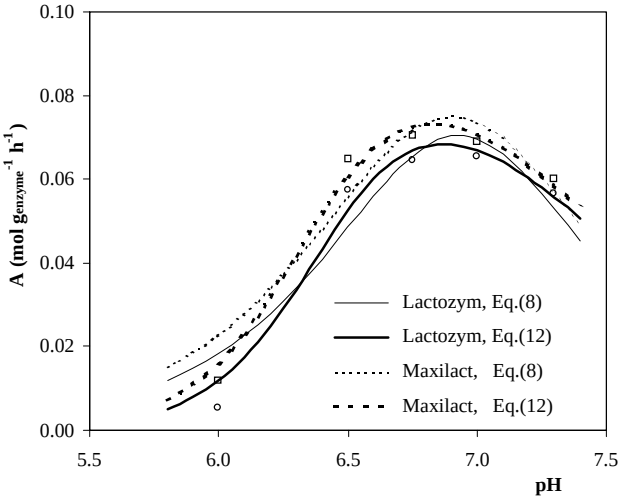

Fig. 3. Influence of pH on Lactozym and Maxilact activity. (○) Lactozym, (□) Maxilact.

The model proposed was applied to the experimental data of activity versus pH obtained by other researchers with β-galactosidases in free state as well as immobilised (Fig. 1; [16] and Fig. 2; [25]). The two proposed models acceptably reproduce the experimental results. The parameters are shown in Table 2, and the r^2 value indicate that there were no significant differences between the two models.

3.2. Thermal deactivation of β-galactosidases

Figs. 4 and 5 show the relative activity versus time for Lactozym and Maxilact at different temperatures, both in Buffer K^+ and Mg^{2+} as well as in Buffer Na^+. The relative activity is defined as:

$$a = \frac{A_t}{A_0} \tag{13}$$

where for each temperature analysed, A_t is the enzymatic activity and A_0 is the initial enzymatic activity.

In the activity experiments conducted at 30 and 35 °C in Buffer K^+ and Mg^{2+}, it was found that both enzymes preserved practically all their activity after 3 h of analysis, while at higher temperatures both enzymes underwent appreciable deactivation (Fig. 4). When the reaction medium was Buffer Na^+, their activity declined rapidly with temperature and time, becoming practically null after 1 h at 40 °C (Fig. 5).

Table 2
Kinetic parameters in the models proposed

Author	Enzyme source/buffer	Kinetic model proposed	pK_1	pK_2	V_{max}	r^2
This work	*Kluyveromyces fragilis* (sol.)/buffer with K^+ and Mg^{2+} ions	Eqs. (5) and (6)	7.40	6.46	0.486[a]	0.889
		Eqs. (9) and (10)	12.8	7.47	9.42×10^{-2}[a]	0.970
	Kluyveromyces lactis (sol.)/buffer with K^+ and Mg^{2+} ions	Eqs. (5) and (6)	7.07	6.74	0.293[a]	0.900
		Eqs. (9) and (10)	12.7	7.49	9.72×10^{-2}[a]	0.980
[16]	*Kluyveromyces fragilis* (sol.)/phosphate buffer with K^+ and Mg^{2+} ions and ONPG	Eqs. (5) and (6)	5.87	7.64	20.5×10^{-5}[b]	0.997
		Eqs. (9) and (10)	11.6	7.77	17.0×10^{-5}[b]	0.970
[25]	*Kluyveromyces fragilis* cells (imm.)/phosphate buffer with K^+ and Mn^{2+} ions and lactose	Eqs. (5) and (6)	6.46	6.99	2.28[c]	0.955
		Eqs. (9) and (10)	12.0	7.38	1.30[c]	0.967

sol.: soluble enzyme, imm.: immobilized enzyme.
[a] $mol_{glucose}\ g_{enzyme}^{-1}\ h^{-1}$.
[b] $mol_{ONPG}\ l^{-1}\ min^{-1}$.
[c] Dimensionless.

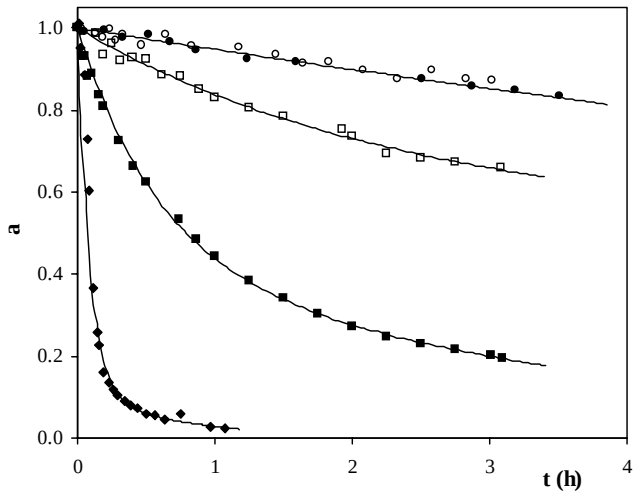

Fig. 4. Fit of the proposed model to the relative activity vs. temperature. Buffer K^+ and Mg^{2+}. Lactozym: (○) 40 °C, (□) 42.5 °C, (■) 45 °C, (◆) 50 °C and Maxilact: (●) 40 °C. (—) Model proposed.

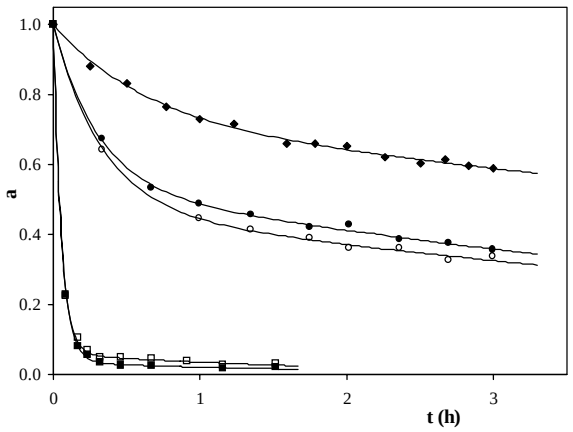

Fig. 5. Fit of the proposed model to the relative activity vs. temperature. Buffer Na^+. Lactozym: (◆) 25 °C, (○) 30 °C, (□) 40 °C and Maxilact: (○) 30 °C, (■) 40 °C. (—) Model proposed.

To explain the behaviour of the enzyme with temperature, we tested different deactivation models that could explain the data: a first-order deactivation model, a second-order deactivation model and different deactivation models with reversible and irreversible reactions in which enzymatic species have different specific activity from the native state [17]. However, the kinetic constants found with the models that fit the experimental results, did not respond satisfactorily to the Arrhenius equation.

We propose a deactivation model:

$$E \underset{k_{ih}}{\overset{k_h}{\rightleftarrows}} E_h \xrightarrow{k_d} E_d \tag{14}$$

where k_h, k_{ih} and k_d are individual kinetic constants of the reaction. This model implies that the native enzyme transforms into a non-active or less active damaged form (E_h) although it can transform again into the native enzyme. Afterwards E_h is denaturalised irreversibly to E_d.

In agreement with this mechanism, the deactivation-kinetic model could be expressed as:

$$\frac{d[E]}{dt} = -k_h[E] + k_{ih}[E_h] \tag{15}$$

$$\frac{d[E_h]}{dt} = k_h[E] - (k_{ih} + k_d)[E_h] \tag{16}$$

$$t = 0, \qquad [E] = [E]_0, \qquad [E]_h = 0 \tag{17}$$

Introducing dimensionless variables:

$$b = \frac{[E]}{[E]_0}, \qquad c = \frac{[E_h]}{[E]_0}, \qquad \tau = k_h\, t \tag{18}$$

the system takes the form:

$$\frac{db}{d\tau} = -b + K_h c \tag{19}$$

$$\frac{dc}{d\tau} = b - (K_h + K_d)c \tag{20}$$

$$\tau = 0, \qquad b = 1, \qquad c = 0 \tag{21}$$

Table 3
Parameters k_h, K_h and K_d calculated by applying the model proposed in Eq. (14)

Author	Buffer	Enzyme source	T (°C)	k_h (h^{-1})	K_h	K_d
This work	Buffer K^+ and Mg^{2+} [a]	*Kluyveromyces fragilis* (sol.)	40	5.07×10^{-2}	2.00	3.27
			42.5	0.200	1.35	0.787
			45	1.08	0.680	0.613
			50	10.3	9.52×10^{-2}	0.147
		Kluyveromyces lactis (sol.)	40	5.07×10^{-2}	2.00	3.27
	Buffer Na^+ [a]	*Kluyveromyces fragilis* (sol.)	25	0.490	1.99	0.513
			30	1.66	0.784	0.144
			40	18.0	5.99×10^{-2}	3.08×10^{-2}
		Kluyveromyces lactis (sol.)	30	1.62	0.990	0.177
			40	17.3	3.59×10^{-2}	3.31×10^{-2}
Fig. 2; [9]	Phosphate buffer	*Aspergillus niger* (imm.)	55	3.64×10^{-3}	2.77	0.711
Fig. 1; [16]	Buffer with K^+, Mg^{2+}, Na^+ and Ca^{2+} ions	*Kluyveromyces fragilis* (sol.)	40	3.92×10^{-2}	11.1	0.634
			45	0.208	0.571	0.206
			50	1.85	0.109	0.130
Fig. 2; [26]		*Kluyveromyces lactis* (sol.)	45	0.875	1.02	0.355
		Kluyveromyces lactis (imm.)	45	1.37	0.964	5.34×10^{-2}

sol.: soluble enzyme; imm.: immobilized enzyme.
[a] Buffer defined in this work.

where,

$$K_h = \frac{k_{ih}}{k_h}, \qquad K_d = \frac{k_d}{k_h} \tag{22}$$

If only the native enzyme is active, the relative activity (a) is given by $a = b$, and if the damaged form retains residual activity (α) by:

$$a = b + \alpha c \tag{23}$$

The model proposed can be compared to the experimental results by numerical integration of the system defined by Eqs. (19)–(21). The best results are found considering that the damaged enzyme is inactive, and therefore $\alpha = 0$. As the deactivation shown for Lactozym and Maxilact is similar in Buffer K^+ and Mg^{2+}, the kinetic treatment of the data at each temperature was made jointly.

Table 3 shows the parameters k_h, K_h and K_d, calculated on applying the proposed kinetic model to the kinetic data for the two buffers used, and Figs. 4 and 5 reflect the fit of the model to the experimental results. Meanwhile, Figs. 6 and 7 present the temperature dependence of k_h, K_h and K_d. Eqs. (24)–(26) are proposed to calculate k_h, K_h and K_d when Buffer K^+ and Mg^{2+} is used:

$$k_h = 7.8 \times 10^{73} \exp\left(-\frac{54200}{T}\right), \quad r^2 = 0.994 \tag{24}$$

$$K_h = 4.09 \times 10^{-44} \exp\left(\frac{31600}{T}\right), \quad r^2 = 0.967 \tag{25}$$

$$K_d = 5.06 \times 10^{-41} \exp\left(\frac{29300}{T}\right), \quad r^2 = 0.948 \tag{26}$$

For verification, the proposed model to explain the thermal deactivation of the β-galactosidases studied was applied to the experimental data proposed by different authors. Table 3 shows the k_h, K_h and K_d values calculated from the data proposed by [9,16] (who used a buffer with K^+ and Mg^{2+} ions) and [26]. The fit of the model to the experimental data is shown in Fig. 8, verifying that the model closely fit the experimental data for the β-galactosidases, both for free-state and immobilised enzymes.

3.3. Influence of lactose on enzymatic activity

To test the influence that the presence of lactose has on the enzymatic deactivation, 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.

Fig. 9 shows the conversion, x (defined as the quantity of lactose hydrolysed divided by the quantity of initial lactose)

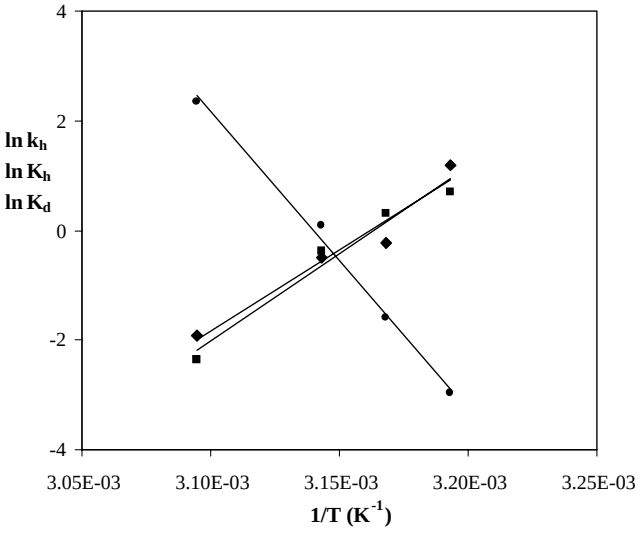

Fig. 6. Temperature dependence of k_h, K_h and K_d. Buffer K^+ and Mg^{2+}. Lactozym and Maxilact. (●) k_h, (■) K_h, (◆) K_d.

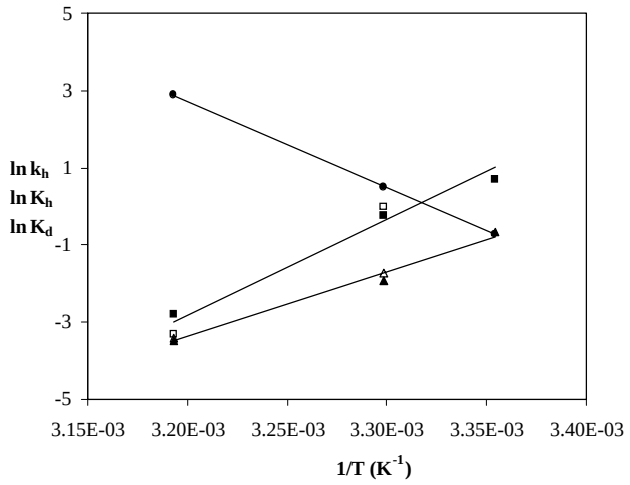

Fig. 7. Temperature dependence of k_h, K_h and K_d. Buffer Na^+ (circle = k_h, square = K_h, triangle = K_d; filled symbols: Lactozym; empty symbols: Maxilact).

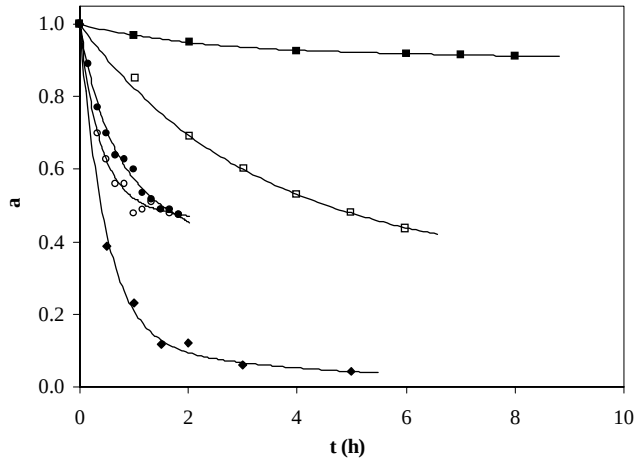

Fig. 8. Fit of the proposed deactivation model to the experimental data obtained by different authors [16] (soluble enzyme): (■) 40 °C, (□) 45 °C, (◆) 50 °C [26], (●) 45 °C, soluble enzyme, (○) 45 °C, immobilized enzyme.

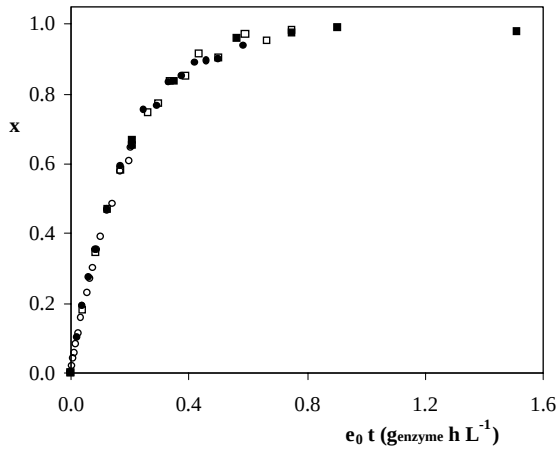

Fig. 9. Influence of lactose concentration on Lactozym activity. Buffer K^+ and Mg^{2+}. Lactose concentration = 25 g l^{-1}, 40 °C. e_0: (○) 0.1 g l^{-1}, (●) 0.5 g l^{-1}, (□) 1.0 g l^{-1}, (■) 3.0 g l^{-1}.

versus e_0t (product of the initial enzyme concentration and time). The experimental results fit the same line, and therefore it appears that lactose stabilises the enzyme according to the postulate by [19–21,27]. Therefore, the enzyme does not undergo enzymatic deactivation in the experimental range tested after lactose hydrolysis.

4. Conclusions

The enzymatic activity of Lactozym and Maxilact present similar behaviour with different pH and temperature values and similar kinetic parameter values. This suggests that both enzymes are probably the same.

Kinetic models were proposed to predict the activity of β-galactosidase versus pH and temperature. The models proposed have been confirmed with the experimental results in the present work as well as of other researchers. The dependence of kinetic parameters versus pH and temperature was determined.

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