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KINETIC MODELING OF THE ANION-EXCHANGE PROCESS OF GLUCOAMYLASES I AND II FROM *ASPERGILLUS NIGER* IN BATCH STIRRED TANK

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ABSTRACT

Purification of high added value products obtained by fermentation processes is a key research task in current biotechnology, especially the ones concerning proteins of very similar molecular structure such as isoenzymes. Purification and separation of biomolecules by commercial ion exchangers is an attractive alternative to affinity chromatography due to its availability and lower cost. For this reason and for its high uptake capacity there is an increasing interest in the application of ion exchangers in biotechnological downstream processing.

In this paper, the kinetics of ion exchange of the two isoenzymes of glucoamylase from *Aspergillus niger* on the anion exchanger DEAE-Toyopearl 650 have been investigated in a batch stirred tank. The experimental results were fitted to a mathematical model accounting for both external fluid film mass

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transfer resistance and pore diffusivity. In addition, the kinetic parameters involved in the process have been obtained for each isoenzyme.

Key Words: Glucoamylase; Isoenzymes; Anion exchange; Modeling; Stirred tank

INTRODUCTION

Nowadays, most enzymatic products are obtained by fermentation using microorganisms, rather than from animal or vegetal sources. Efficient isolation and purification of a biological product from its fermentation broth is essential for commercial success, since downstream recovery means a large portion of the product cost, particularly for complex molecules (1). Starting from a broth where the product of interest is present at very low concentration and the main contaminants are generally compounds of similar structure, size, and physicochemical properties than those of the target enzyme, a high-resolution technique must be used to carry out the final steps in the purification process. Ion exchange chromatography is often used as such a technique because the biological activities of these proteins are not significantly affected (2) due to the mild operating conditions employed and its relatively low cost compared with affinity chromatography.

In a system where a solution of adsorbate is brought into contact with a macroporous solid adsorbent, the kinetics of adsorption or ion exchange are governed by the external mass transfer resistance and the intraparticle diffusivity, characterized by the external fluid film mass transfer coefficient and the pore diffusion coefficient, respectively. While studying a particular ion exchange process, slurry adsorbents are useful in batch processing of fluids and determining parameters for the further design and operation of fixed bed adsorbers (3). Important advantages of these systems stem from the fact that external mass transfer resistance can be lowered by increasing the agitation power input. Under these conditions, intraparticle diffusivity is expected to be the main mass transfer resistance.

In addition, the extent and mechanism of the adsorbent–adsorbate equilibrium reached in this process must be taken into account in order to perform an accurate and rigorous modeling of the data obtained from the kinetic experiments.

The present study deals with the kinetics of the ion exchange process of the two isoenzymes of glucoamylase from *Aspergillus niger* (named Glucoamylase I and Glucoamylase II) on the anionic macroporous resin DEAE-Toyopearl 650 using a batch stirred tank system. A phenomenological mathematical model

including pore diffusivity as the main transfer-controlling step was proposed and used to study the adsorption of both isoenzymes. The effect of the main operating variables (temperature and initial concentration of protein) on the kinetic behavior of each glucoamylase isoenzyme has been studied. Data were obtained for the transport of the two isoenzymes into the resin. Measurements were made at 25, 20, and 15°C and at three particle diameters. The mathematical model proposed described adequately the adsorption of both isoenzymes on DEAE-Toyopearl 650 resin.

MATHEMATICAL MODEL

The basic rate mechanism of ion exchange of macromolecules on the internal porous surface of an adsorbent is considered to occur by three processes in series: (i) transfer of molecules across the stagnant liquid film surrounding the particles, (ii) diffusion inside the pores, and (iii) adsorption by ion exchange onto the surface. Diffusion of proteins on the solid phase of an anion exchanger has been reported scarcely in the literature. However, the cases reported were dealing with systems with a very strong interaction between the molecule of adsorbate and the ligands of the adsorbent. So, in this study, surface diffusion has not been accounted for the development of the mathematical model.

The above mechanism can be implemented into a kinetic model. The mathematical equations that describe the transient behavior of batch stirred tank systems have been formulated assuming that the following physical steps take place simultaneously during adsorption:

(a) external fluid film mass transfer,
(b) mass transfer within particle pores by macropore diffusion, and
(c) the rate of adsorption by ion exchange is much faster than the rate of diffusion (local equilibrium at the surface of the pores).

For the sake of simplicity, a few basic assumptions have been made:

(1) the resin particles are spherical and of uniform size and density,
(2) the conditions of adsorption equilibrium process by ion exchange mechanism (temperature, pressure, and concentration) at all points around the surface of a given particle are the same,
(3) the changes in densities of both fluid and the solid are small throughout the adsorption process,
(4) the transport of isoenzyme molecules into the porous adsorbent is defined by Fickian diffusion with an effective pore diffusion coefficient, D_p, and
(5) mass transfer to the surface of the adsorbent is governed by a film model characterized by a mass transfer coefficient, k_f.

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In this model, the concentration of isoenzyme within the particle pores is time and radius dependent, and is described by the following equation:

$$\varepsilon_p \frac{\partial C_p}{\partial t} + (1 - \varepsilon_p)\frac{dq}{dt} = \varepsilon_p D_p \left(\frac{\partial^2 C_p}{\partial r^2} + \frac{2}{r}\frac{\partial C_p}{\partial r} \right) \quad (1)$$

From the second assumption, the equilibrium of adsorption can be expressed by a general isotherm equation, where C_p is a function of q:

$$q = f(C_p) \quad (2)$$

The boundary conditions at the external surface and at the center of the particle are given by Eqs. (3) and (4), respectively.

$$-D_p \varepsilon_p \left(\left. \frac{\partial C_p}{\partial r} \right|_{r=R} \right) = k_f \varepsilon_p (C - C_p|_{r=R}) \quad (3)$$

$$\left. \frac{\partial C_p}{\partial r} \right|_{r=0} = 0 \quad (4)$$

The flux of protein towards the surface of the resin particles through the external fluid film causes a concentration depletion of the solution in the batch stirred tank according to:

$$V_L \frac{dC}{dt} = \frac{3 k_f V_p}{R} \varepsilon_p (C - C_p), \qquad (r = R) \quad (5)$$

The initial conditions for the above equations are:

$$C_p = 0, \qquad (r = 0;\ t = 0) \quad (6)$$

$$q = 0, \qquad (r = 0;\ t = 0) \quad (7)$$

$$C = C_0, \qquad (t = 0) \quad (8)$$

The application of non-linear adsorption isotherms requires the use of numerical methods to solve the above mass balance equations. A flexible numerical method was used to solve the problem and a semidefinite form of the finite elements method was chosen to reduce the partial differential equations to a set of ordinary differential equations. The equations were discretized only for the radial position. The algorithm used for solving this stiff set of ordinary differential equations was implemented in a Fortran software using the subroutine D02EBF from the NAG Fortran Library (Numerical Algorithms Group Inc., Downers Grove, IL, USA), which integrated the differential

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equations using a variable-order, variable step Gear method. The above Eqs. (1)–(8) were solved numerically to give predicted concentration vs. time curves in terms of D_p. The values to give the closest agreement with experimental results were selected in order to minimize the error function (Eq. (9)), constructed from the predicted and experimental kinetic curves.

$$\Phi = \frac{\sum_{i=1}^{n} (C/C_0|_{exp} - C/C_0|_{theo})^2}{n} \tag{9}$$

EXPERIMENTAL

Materials

All the studies reported here were performed using acetate buffer. According to a previous work (4), 20 m*M* acetate buffer of pH 5.5 and ionic strength of 0.18 *M* was prepared by dissolving known quantities of analytical grade sodium acetate, acetic acid, and NaCl in distilled and deionized water in the required amounts (5). Every solution was filtered through 0.80 μm cellulose ester membranes (AAWP04700, Millipore, Bedford, MA, USA) prior to use.

AMG 300L containing Glucoamylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.3) was supplied by Novo Nordisk Bioindustrial, S.A. (Madrid, Spain). Then, Glucoamylase I (GA I) and glucoamylase II (GA II) were isolated and purified according to a protocol developed in our laboratory (4). Low initial concentrations of protein were employed in the buffered bulk solutions (0.30, 0.60, and 1.30 g/L for GA I, and 0.25, 0.50, and 1.05 g/L for GA II) in order to simulate the glucoamylase concentration found in an industrial fermentation broth. Furthermore, the initial concentration of every glucoamylase was not the same because GA I is produced in higher amounts than GA II.

The anion exchanger DEAE-Toyopearl 650 was purchased from TosoH Corp. (Tokyo, Japan). Before use, 100 mL of resin suspension was filtered, washed with 1 L distilled deionized water and dried at 70°C overnight. Afterwards, the anion exchanger was sieved and the fraction 63–100 μm was employed due to its better performance observed in previous empirical tests. The properties of this ion exchanger are summarized in Table 1.

Apparatus

The experimental device used to carry out the batch stirred tank anion exchange experiments consisted of a 5 cm diameter Pyrex spherical vessel where

Table 1. Physicochemical Characterization of DEAE-Toyopearl 650

Particle porosity	0.73
Average particle size (μm)	81.5
Solid phase density (g/cm^3)	1.47
Particle density (g/cm^3)	0.77
Ion exchange capacity (meq/g)	0.25
BET surface area (m^2/g)	43.1
Average pore volume (cm^3/g)	0.62

the protein solution was placed and mixed with the resin particles by means of a mechanical mixer impeller (Ika RW-20, Janke and Kunkel, Staufen, Germany). The temperature of the system was kept constant in every experimental run by immersing the stirred tank in a thermostatic bath (Heto DT1 CB-8-30e, Heto-Holten, Allerød, Denmark).

The protein solution was recirculated from the vessel using tygon tubing connected to a Microperpex S peristaltic pump (Pharmacia LKB, Uppsala, Sweden). The protein concentration in the liquid phase was measured through the UV absorbance at 280 nm using a flow cell fitted to an UV-M-II ultraviolet detector (Pharmacia LKB, Uppsala, Sweden) and continuously monitored on a Rec-2 chart recorder (Pharmacia LKB, Uppsala, Sweden). The dead time of the liquid passing through the recirculation system was less than 6 sec. A 10 μm Teflon filter was used at the entrance of the tygon tube to avoid resin particles leaving the vessel.

Preliminary Experiments

Three separate experiments were performed with an agitation rate of 400, 700, and 1000 rpm, respectively. The ion exchange uptake rates observed were identical, showing the reproducibility achieved, indicating that the resin particles were fully fluidized with no sign of damage within the experimental range. The impeller speed was set at 700 rpm. Three experiments were performed under identical operating conditions but varying particle diameter ranges (20–50, 63–100, and 100–150 μm) to select the experimental range studied. No significant differences were observed in the optimum value of the pore diffusion coefficient in these experiments and the range 63–100 μm was used. Another series of experiments were performed in order to set the volume of adsorption solution and amount of resin. It was concluded that 40 mL and 0.5 g of dried resin, respectively, were proper values for experimentation, due to the homogeneity and

reproducibility achieved. The kinetic studies were carried out at three different temperatures (15, 20, and 25°C) due to the high stability of both isoenzymes within that experimental range (6).

RESULTS AND DISCUSSION

Adsorption isotherms of both the glucoamylase isoenzymes on the anion exchanger DEAE-Toyopearl 650 are shown in Fig. 1. The equilibrium of adsorption corresponding to GA I followed a marked non-linear behavior that was satisfactorily described by the Langmuir isotherm equation. However, the equilibrium of adsorption of GA II was linear within the experimental range and it can be fitted to Henry's law (7). The ion exchange capacity of GA II is significantly lower than that of GA I.

The kinetic uptake curves (Fig. 2) show the dimensionless concentration in the bulk liquid phase as a function of time for the adsorption of pure isoenzymes on the anion exchanger. For both adsorbates, the adsorption capacity of the resin increased as temperature increased according to the results obtained from the experimental isotherms. In addition, the initial slope of the uptake curves increased with temperature. This positive effect on the extent of the ion exchange equilibrium has been reported previously for other proteins (14–16). Protein configuration or reorientation and water release from the surface can lead to an entropically driven endothermic ion exchange processes (15). Since the external film mass transfer resistance was removed, the above behavior suggested that internal diffusion, favored by temperature, governed the kinetics of the process. In addition, it can be seen (Fig. 2) that the equilibrium of adsorption was faster for GA II than for GA I, due to the smaller size of the former molecule, producing a faster diffusion across the pores.

On the other hand (Fig. 3), as initial concentration increased, the amount of both isoenzymes adsorbed on the resin also increased as predicted by the isotherm equations. Furthermore, for higher values of C_0, the equilibrium was reached faster since the driving force along the pores was higher.

Kinetic Modeling

The mathematical model described was used to fit the experimental kinetic curves for the uptake of both GA I and GA II at different operating conditions (Fig. 4). The best fitting mass transfer parameters, D_p and k_f, were obtained by minimization of the function Φ (Eq. (8)). The optimum values of k_f, and so the Sherwood numbers, were high in all the experiments. These results showed that the kinetics of the process was not under the control of the external film mass

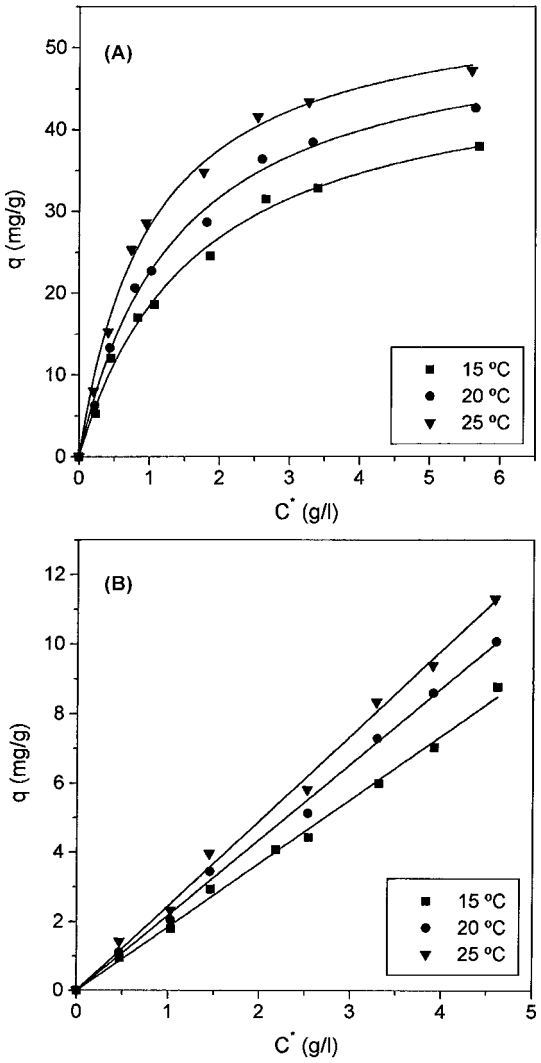

Figure 1. Adsorption isotherms of Glucoamylase I (A) and Glucoamylase II (B) on DEAE-Toyopearl 650 ion exchanger.

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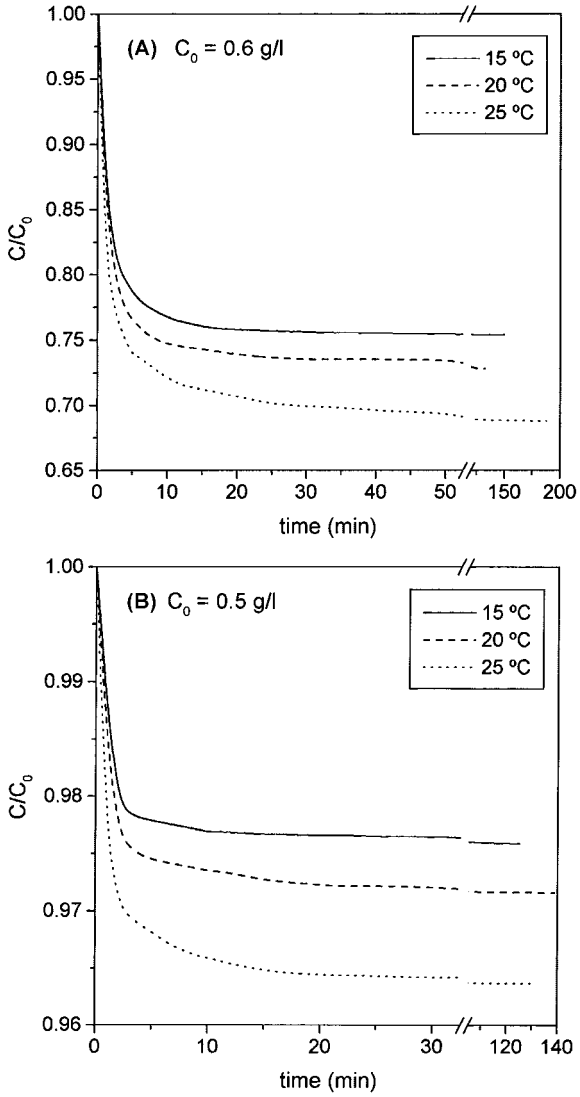

Figure 2. Experimental uptake curves of GA I (A) and GA II (B) at different temperature.

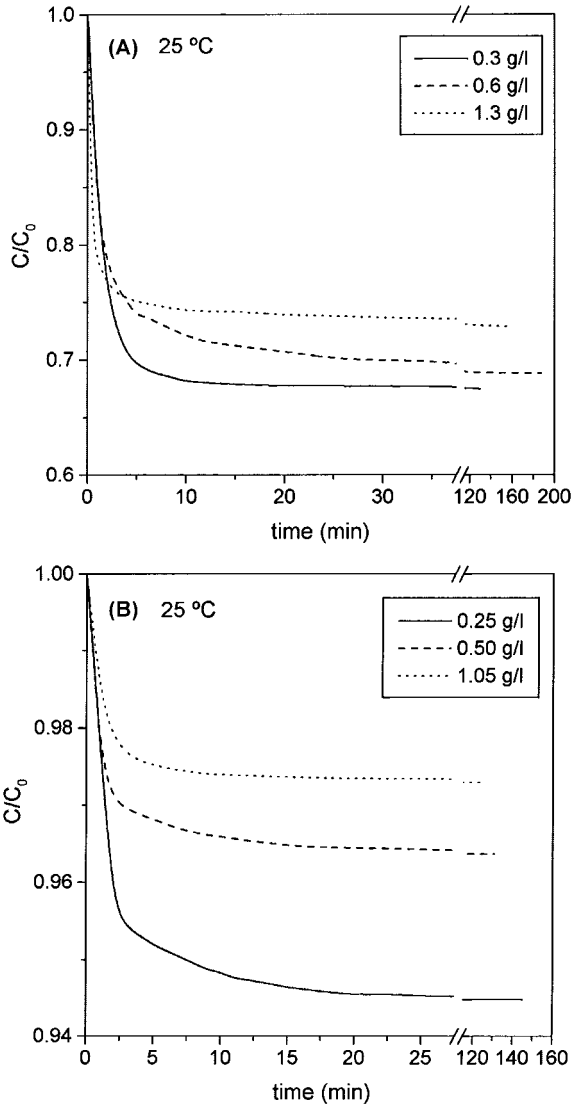

Figure 3. Experimental uptake curves of GA I (A) and GA II (B) at different initial concentrations.

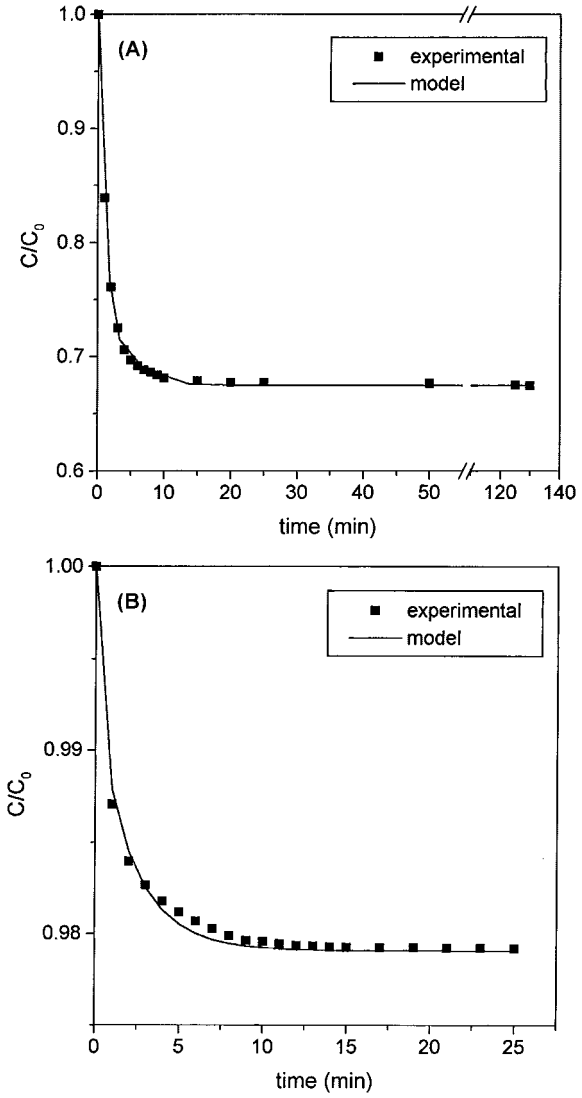

Figure 4. Kinetic modeling of experimental curves of GA I (A) and GA II (B). (A): $T = 25°C$, $C_0 = 0.30\,kg/m^3$; (B): $T = 15°C$, $C_0 = 1.05\,kg/m^3$.

transfer resistance, confirming the choice of the stirring speed during previous experiments.

The internal diffusion was the controlling mass transfer mechanism during the process and this was noticed also during the parameter optimization. Here, increasing values of the external film mass transfer coefficient yielded lower values of the objective function. However, for larger k_f values, the objective function asymptotically approached to a minimum constant value because the external mass transfer was no longer a controlling mechanism in that region.

The low calculated values of D_p (Table 2) indicated that there was an important restriction to the flux of adsorbate along the pores due to the high molecular weight of GA I and GA II, 83 and 71 kDa, respectively (8).

The molecular diffusivity in free solution, D_0, of both glucoamylases was estimated by the Polson equation (9). For each isoenzyme, the ratio D_0/D_p (also named tortuosity factor) was, approximately, constant over the experimental

Table 2. Model Parameters of the Experiments Performed with GA I and GA II on DEAE-Toyopearl 650

T (°C)	C_0 (g/L)	D_0 ($\times 10^{-11}$ m^2/sec)	D_P ($\times 10^{-11}$ m^2/sec)	$\bar{D}_p$ ($\times 10^{-11}$ m^2/sec)	τ	Φ ($\times 10^5$)
GA I						
	0.30		1.14			0.24
15	0.60	5.45	1.20	1.19	4.6	0.63
	1.30		1.22			0.49
	0.30		1.30			0.60
20	0.60	6.31	1.33	1.33	4.8	1.16
	1.30		1.35			0.16
	0.30		1.87			0.49
25	0.60	7.22	1.62	1.69	4.3	0.25
	1.30		1.59			0.46
GA II						
	0.25		2.30			1.32
15	0.50	5.74	1.71	1.90	3.1	0.78
	1.05		1.68			3.41
	0.25		2.70			0.40
20	0.50	6.64	2.51	2.52	2.7	0.89
	1.05		2.35			0.74
	0.25		3.04			0.98
25	0.50	7.60	2.70	2.72	2.8	0.58
	1.05		2.43			0.11

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temperature range studied. This suggested that the pore diffusion was the main mass transfer mechanism inside the pores. Similar behavior has been reported for the adsorption of other proteins on macroporous resins (10,11). However, the fitting values of D_p for GA II showed a slightly increasing trend as C_0 increased. This may be due to the very low adsorption capacity of GA II on DEAE-Toyopearl at low C_0. Then, the relative experimental error was larger than that found for higher adsorbate concentration, resulting in higher uncertainty in the optimum value of the pore diffusion coefficient.

GA I is composed of two domains with approximately spherical symmetry, connected to each other through a long chain of 38 amino acid residues, yielding a very long and asymmetric rigid structure (12). On the other hand, GA II mainly consists of one of the globular domains of GA I (13). Thus, the difference in the relative effective dimension between both isoenzymes produces a more severe restriction to the flux inside the pores for GA I due to steric hindrance. This explained the significantly larger value of tortuosity factor obtained for GA I (Table 2).

CONCLUSIONS

In order to study the effect of temperature and initial concentration of protein on the ion exchange processes, a series of experiments was carried out in a batch stirred tank system using Glucoamylase I and Glucoamylase II as adsorbates and DEAE-Toyopearl 650 resin as anion exchanger.

A mathematical model was proposed to predict the experimental kinetic curves of the anion exchange systems described above. It was concluded that the model was adequate to predict the kinetic behavior of the system within the experimental range studied. The results showed that pore diffusion was the main resistance to mass transfer in the process.

In addition, the tortuosity factor arises as an important factor in the transport properties of macromolecules inside the macropores of the adsorbent particles because it accounts for differences in the molecular geometry of the macromolecule. In the present work, the D_0/D_p ratio for GA I, a highly asymmetric molecule, was larger than that of GA II, with a higher spherical symmetry. The above difference in the D_0/D_p ratio between both of the isoenzymes was larger than expected from molecular size and molecular weight considerations.

The mathematical model can be used to simulate the ion exchange process in order to evaluate the kinetic behavior at different operating conditions for both design and scientific purposes. The estimated values of pore diffusion coefficient, obtained from the application of the model, could be used in a further step to simulate, design, and scale-up the ion exchange process of both isoenzymes of glucoamylase at industrial scale.

NOMENCLATURE

C	adsorbate concentration in the bulk liquid phase
C_0	initial adsorbate concentration in the bulk liquid phase
C_p	adsorbate concentration in the liquid phase inside the pores
C^*	equilibrium concentration of adsorbate in the liquid phase
D_p	pore diffusion coefficient
D_0	molecular diffusivity
k_f	external film mass transfer coefficient
n	number of experimental data points
q	equilibrium adsorbate concentration in the solid phase
r	radial position inside the particle
R	average particle radius
Sh	Sherwood number
t	time
T	temperature
V_L	volume of the liquid phase
V_p	pore volume

Greek letters

ε	bed voidage
ε_p	particle porosity
Φ	error function (Eq. (9))
τ	tortuosity factor (= D_0/D_p)

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