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Expanded-Bed Adsorption Utilizing Ion-Exchange Resin to Purify Extracellular β-Galactosidase

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ABSTRACT

The application of expanded-bed ion-exchange resins allows the elimination of intermediary particulate separation steps like filtration or centrifugation prior to adsorption steps in enzyme-purification processes from crude fermentation broths. This work is concerned with the experimental evaluation data of a process related to the adsorption of an extracellular β-galactosidase from the fungi *Scopulariopsis*. The protein recovery in the ion-exchange resin Accell Plus QMA™ was accomplished using a continuous-monitoring method. The direct adsorption step was followed by a elution step with concentrated NaCl solutions aiming to improve the enzyme-specific activity. Experimental data for fixed and expanded bed were compared.

Index Entries: Extracellular β-galactosidase; adsorption; expanded bed; enzyme recovery.

INTRODUCTION

The preservation of molecular integrity and the pursuit of high-product yield during recovery and purification operations is of paramount concern to the bioprocess engineer. This is best accomplished by the elimination of unnecessary processing operations. The initial steps of the recovery and purification train typically involve time-consuming and inefficient

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solid-liquid separation stages to generate the particulate-free feedstream required for subsequent chromatographic operations. Conventional chromatography is operated in downflow fixed-bed adsorption columns. Because of the small inter-particle voidage between the solid-phase components, a particulate-free feedstream is mandatory for their operation. Such problems can be avoided if the direction of the inlet feed is reversed and the linear flow velocity of the liquid is increased. Inter-particle voidage increases and whole-broth processing becomes a possibility. This operation is named expanded-bed adsorption and has been utilized by several authors for enzyme recovery *(1–4)*.

A strain of *Scopulariopsis* that is a potent producer of extracellular β-galactosidase was isolated by Pastore and Park in 1980 and submitted to some preliminary purification and characterization *(5)*. The low yield (4%) obtained in those procedures motivated the application of the new technique of expanded-bed chromatography with relatively high-density resins. This paper describes the use of a purpose-designed expanded-bed adsorption system for the direct capture of the extracellular enzyme from homogenized fermentation broths and the comparison of this approach with traditional packed-bed routes.

MATERIALS AND METHODS

Production of β-Galactosidase from *Scopulariopsis*

The strain of the fungi *Scopulariopsis* selected previously *(5)* was utilized to produce β-galactosidase in wheat-bran medium (Koji process). After incubation of wheat (that is inoculated with spores) at 30°C for 5 d, 100 mL of deionized water was added to 20 g bran. This was soaked for 1 h at room temperature. The proteic extract containing the enzyme was precipitated by ethanol (70% of final concentration) at 4°C. The precipitate was centrifugated and the solids were subjected to freeze-drying.

Determination of the Protein Concentration

Total protein concentration was monitored trough UV-absorbance by using a medium-pressure chromatographic system (Biologic System, Biorad Richmond, CA). The protein concentration was determined by the Bradford method *(6)* utilizing bovine serum albumin as standard.

Determination of the Enzyme Activity

As proposed by Pastore and Park *(5)*, β-galactosidase activity was determined by incubating a mixture of 10 μ of the enzyme and 0.15 mL of 0.25% w/w solution of the substrate o-nitrophenyl β-D-galactopyranoside (ONPG) for 15 min at 60°C. The buffer utilized for the control of incubation experiments was 1.69 mL of 100 m*M* acetate, pH = 5.0. The product of the reaction is o-nitrophenol (ONP), and the reaction is interrupted by the

addition of 0.15 mL of 10% sodium carbonate solution. One unit of β-galactosidase activity was defined as the amount of enzyme that liberates 1 μmole of ONP per minute under the conditions described above.

EXPERIMENTAL SET-UP FOR ADSORPTION STUDIES

In Fig. 1 the experimental set-up utilized for the adsorption studies, for fixed and fluidized beds is depicted. A glass column from Pharmacia (Uppsala, Sweden) with a diameter of 1 cm and a total height of 20 cm was utilized both for the packed-bed and fluidized-bed experiments. This column has an appropriate distributor and a mobile piston that enables the variation of the bed height. Systems were equilibrated with the Tris-HCl buffer (pH = 7.5 and ionic strength of 0.03 *M*), loaded and washed at a flow rate of 1 mL/min. The typical amount of resin for adsorption and elution tests was 1.0 g, which is equivalent to an initial bed height of 2.6 cm. Elution was carried out using a linear gradient with the same buffer to which NaCl was added to form a 0.75 M NaCl solution. Fractions of 2 mL were collected both during the adsorption and elution procedures.

Characterization of the Resin Beds

The adsorbent Accell Plus QMA is an anion-exchange resin commercialized by Waters, a division of Millipore (Bedford, MAP). This resin has a core of silica covered by an acrylamide copolymer containing quaternary amine functional groups. The range of particle size is from 37 to 55 μ and the density when equilibrated in the buffer Tris-HCl, pH = 7.5 and ionic strength of 0.03 *M* is 1.18 g/mL, as determined by picnometry. These physical properties are convenient for experiments involving expanded beds at low flow rates.

The determination of bed structural characteristics such as bed porosity and particle porosity is essential for scale-up calculations. Aiming at the experimental evaluation of those parameters, a pulse method using the moments theory was developed by Arnold et al. *(7)* and utilized in the present work. The principles of the method involve the concentration measurement of a tracer pulse as a function of time, being the concentration C(t) related to the first moment (μ_1) by the equations:

$$\mu_1 = \frac{\left(\int_0^\infty C(t) \times t \times dt\right)}{\left(\int_0^\infty C(t)dt\right)} \tag{1}$$

$$\mu_1 = \left(\frac{L}{u}\right)\left\{\varepsilon + (1 - \varepsilon)\beta\left(1 + \left[\frac{\rho_p}{\beta}\right]K\right)\right\} + \left(\frac{t_0}{2}\right) \tag{2}$$

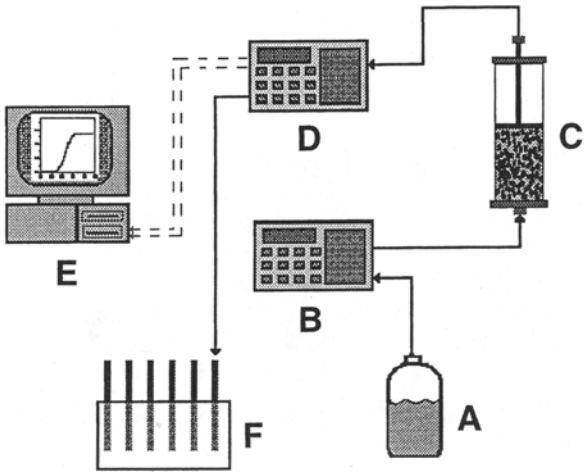

Fig. 1. Scheme of the experimental setup for column adsorption studies. **(A)** Enzyme solution; **(B)** piston pump; **(C)** expanded- or fixed-bed column; **(D)** UV detector; **(E)** data-acquisition system; **(F)** fraction collector.

In equation *(2)*, L is the bed length, u is the fluid superficial velocity, ε is the bed porosity, β is the particle porosity, ρ_p the particles density, t_0 is the injection time, and K is the adsorption constant for a linear isotherm. Therefore the equations above give the relationship among the first moment and bed and hydrodynamics variables.

For the case of a tracer pulse in which the adsorption is avoided (large molecule or high ionic strength of the solution) the above equations can be simplified to:

$$\mu_1 = \left(\frac{L}{u}\right)\{\varepsilon + (1 - \varepsilon)\beta\} + \left(\frac{t_o}{2}\right) \tag{3}$$

The use of a high-molecular-weight polymer (blue dextran) as a tracer avoids the pore penetration by the solute and implies that $\beta = 0$. Combining this fact with experiments carriedout by injection of a low-molecular-weight tracer (acetone), allows the simultaneous determination of β and ε from the above equations, for a given injection time t_0.

RESULTS AND DISCUSSION

Bed Expansion

The measurement of the bed height as a function of the fluid superficial velocity lead to the bed expansion characteristics as shown in Fig. 2. Straight lines as those depicted are classical in fluidization of solids by using liquid *(8)*. Our results shows a slight modification for the original

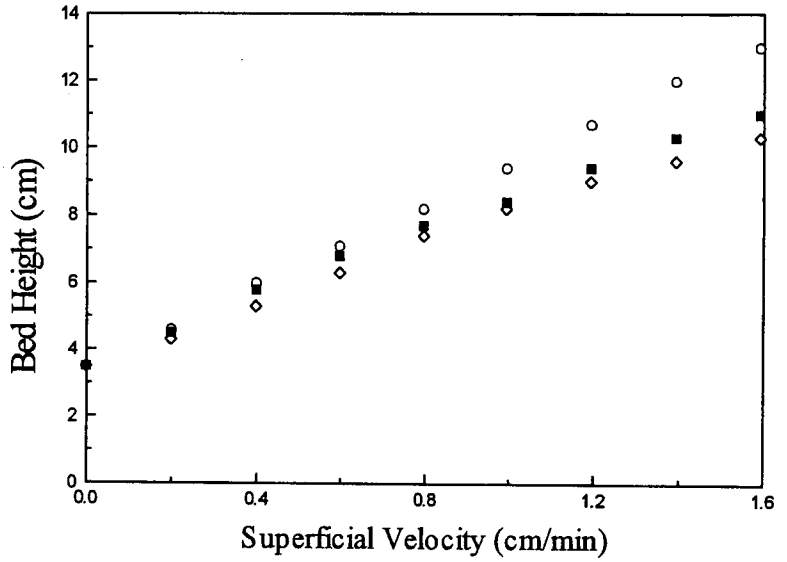

Fig. 2. Effect of superficial velocity on the bed expantion ○ - Original resin; ■ - eluted resin; ◇ - loaded resin.

and used resin particles, caused probably by the variation in particle density and to injection of solutions of slightly different viscosities during the adsorption and elution cycle.

Characterization of the Resin Bed

Concentration of both blue dextran and acetone as a function of time in pulse experiments is depicted in Figs. 3 and 4. From equation *(1)*, the first moment μ_1 can be calculated, and bed porosity, ε, and the particles porosity β, can be obtained from equation *(3)*. A summary of the calculations is depicted in Fig. 5. The experiments conducted with blue dextrane and acetone for the bed constituted by Accell Plus QMA resin particles resulted in the values of 0.41 and 0.59 for ε and β, respectively, which are very characteristic for fixed beds and particles of ion-exchange resins. From the fixed-bed porosity value, the expanded bed porosity could be calculated by using the expansion curve (Fig. 2) and equation *(4)* based on the fluidization theory *(9)*:

$$H_1(1 - \varepsilon_1) = H_0(1 - \varepsilon_0) \quad (4)$$

In equation *(4)* subscript 0 means initial conditions for porosity and bed height and subscript 1 indicates the values for the same variables in expanded bed.

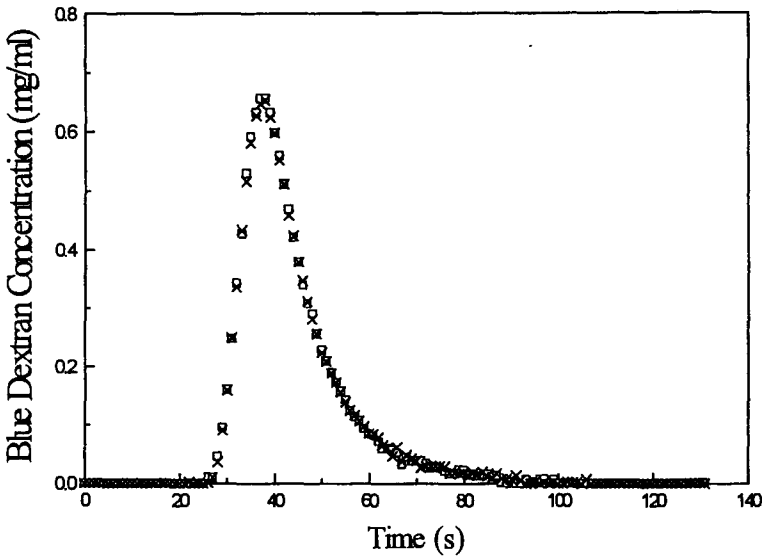

Fig. 3. Response peaks for the injection of 200 μL of a 1.0 mg/mL blue dextran solution. □ - First injection; × - second injection.

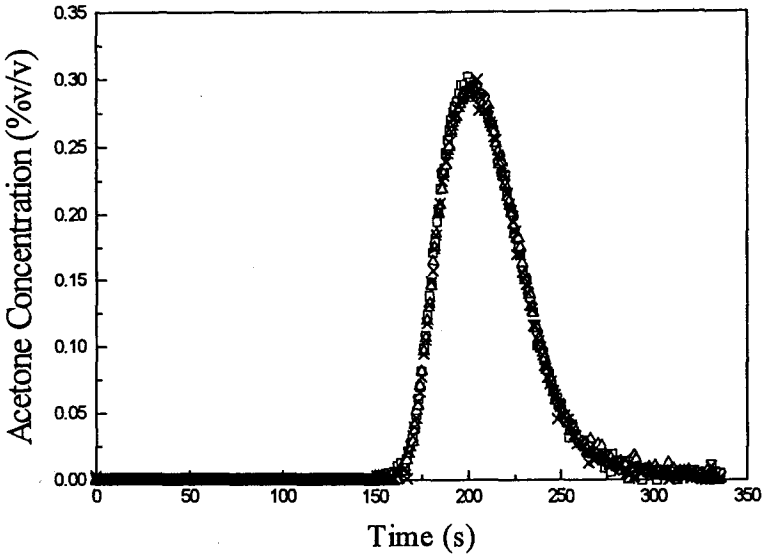

Fig. 4. Response peaks for the injection of 200 μL of a 1% (v/v) acetone solution. □ - First injection; △ - second injection; × - third injection.

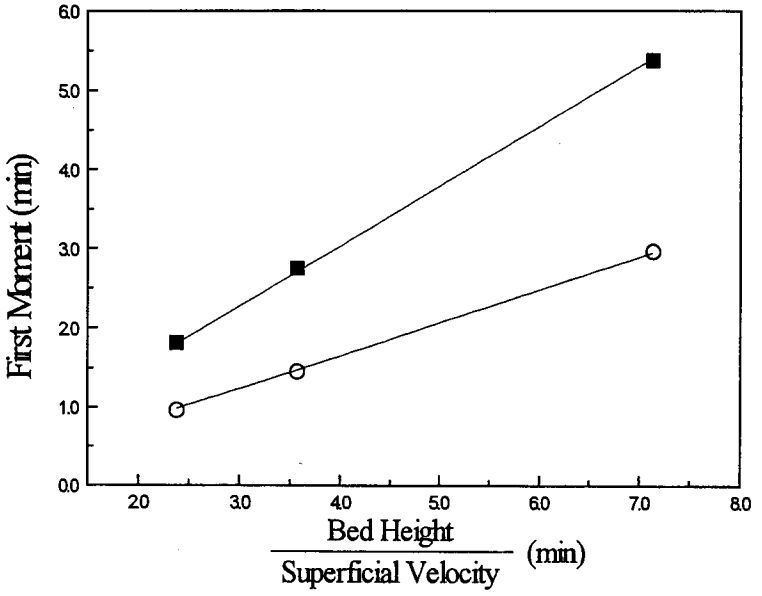

Fig. 5. Determination of bed and particle porosities using statistical moments analysis. ■ - Acetone; ○ - blue dextran.

Recovery of β-Galactosidase: Comparison Between Packed and Expanded Bed Adsorption

The loading flow rate of 1 mL/min, correspondent to a superficial velocity of 1.27 cm/min, resulted in a degree of expansion of approx 3.0. The degree of expansion dropped slightly during loading, but did not require a change in loading flow rate.

The specific activity of the enzyme preparation determined by the assay method described previously was 25.2 U/mg for the packed-bed experiments and 17.2 U/mg for the expanded-bed assays.

Typical chromatograms for the fixed and expanded bed are illustrated in Figs. 6 and 7. In the packed-bed route 92.4% of the total β-galactosidase loaded was recovered compared to 83% for the expanded bed. Both routes led to well-defined sharp β-galactosidase peaks, and Table 1 presents the results from successive loadings onto both the expanded and packed bed in terms of purification factor and yield. Purification factors for the fixed and expanded bed reached values of 3.2 and 2.2, respectively.

CONCLUSIONS

From the experimental procedures carried out, is clear that the direct recovery of extracellular β-galactosidase utilizing an ion-exchange resin utilizing an expanded-bed column is a viable operation. The enzyme yield

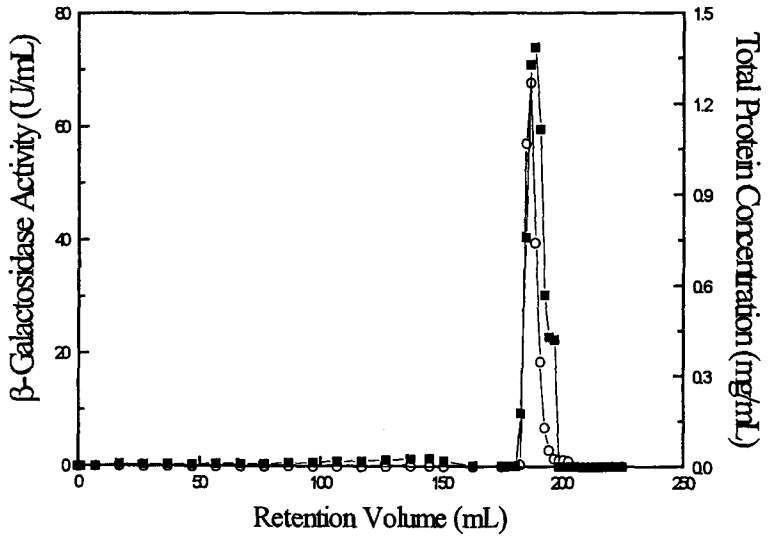

Fig. 6. Chromatogram for β-galactosidase recovery using ion-exchange resin Accell Plus in packed bed. ○ - Enzyme activity; ■ - protein concentration.

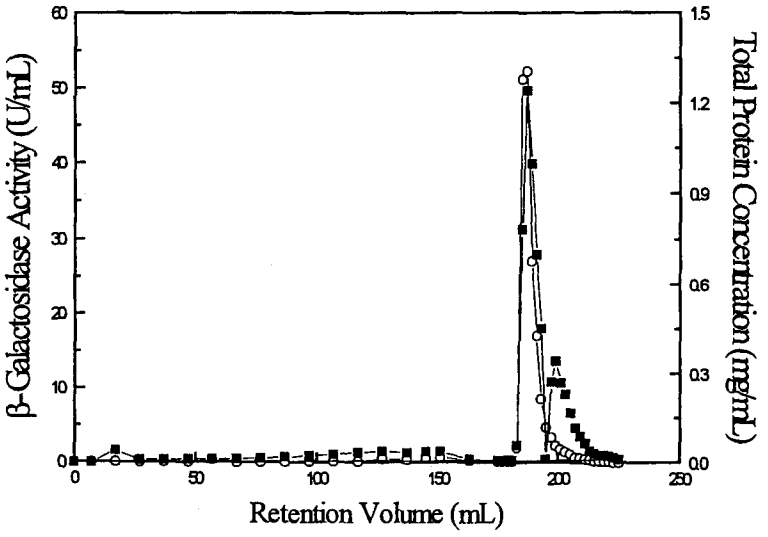

Fig. 7. Chromatogram for β-galactosidase recovery using ion-exchange resin Accell Plus in expanded bed. ○ - Enzyme activity; ■ - protein concentration.

obtained from the expanded-bed system is lower than the packed bed, but both have comparable performances. The purification factors are 3.2 and 2.2, respectively. These results can be enhanced by optimization of the operational conditions such as flow rate, pH, and ionic strength. It should be remembered that packed beds foul more rapidly than expanded beds,

Table 1
Yield and Purification Factors for Packed- and Expanded-Bed Operations

		Volume (mL)	SA[1] (U/mg)	Purification Factor	Total Activity	yield %
Packed Bed	**Initial**	**144**	**25.2**	**1.0**	**396**	**100.0**
	Final	**8**	**79.6**	**3.2**	**366**	**92.4**
Expanded Bed	**Initial**	**144**	**17.2**	**1.0**	**400**	**100.0**
	Final	**14**	**37.3**	**2.2**	**333**	**83.0**

[1] SA - Specific Activity of β-Galactosidase

requiring normal solid-liquid operations before the adsorption step. The additional operation required by packed-bed operations results in advantage for the expanded bed in terms of total processing time economics as well as in terms of global efficiencies.

Utilization of the moments theory enabled the characterization of important bed parameters such us the bed porosity and the particle porosity for the resin Accell Plus QMA.

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