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Production of Cyclodextrins in a Fluidized-Bed Reactor Using Cyclodextrin-Glycosyl-Transferase

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Abstract

Cyclodextrin-glycosyl-transferase (EC2.4.1.19), produced by Wacker (Munich, Germany), was purified by biospecific affinity chromatography with β-cyclodextrin (β-CD) as ligand, and immobilized into controlled pore silica particles (0.42 mm). This immobilized enzyme (IE) had 4.7 mg of protein/g of support and a specific activity of 8.6 μmol of β-CD/(min·g_{IE}) at 50°C, pH 8.0. It was used in a fluidized-bed reactor (FBR) at the same conditions for producing cyclodextrins (CDs) with 10% (w/v) maltodextrin solution as substrate. Bed expansion was modeled by the Richardson and Zaki equation, giving a good fit in two distinct ranges of bed porosities. The minimum fluidization velocity was 0.045 cm/s, the bed expansion coefficient was 3.98, and the particle terminal velocity was 2.4 cm/s. The FBR achieved high productivity, reaching in only 4 min of residence time the same amount of CDs normally achieved in a batch reactor with free enzyme after 24 h of reaction, namely, 10.4 m*M* β-CD and 2.3 m*M* γ-CD.

Index Entries: Cyclodextrin; cyclodextrin-glycosyl-transferase; fluidized-bed reactor; maltodextrin.

Introduction

Cyclodextrins (CDs), also known as Schardinger's dextrins, are nonreducing cyclic oligosaccharides formed by D-glycosyl residues linked by α,1-4 bonds. They are most frequently produced by the action of the enzyme cyclodextrin-glycosyl-transferase (CGTase) on liquefied starch. They usually contain from six to eight glycosyl residues and are called α-, β-, and γ-CD, respectively *(1–3)*.

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Cyclic molecules of CDs contain a hydrophobic cavity that may encapsulate at the molecular level, the nonpolar part of many organic molecules. This usually confers to the complexed molecule higher physical and chemical stability; it may improve the solubility of poorly soluble drugs, and also increase the potency of their biological action. These characteristics have created the opportunity for many industrial applications of CDs in foods, cosmetics, pharmaceuticals, agriculture, chromatographic separations, laundry fragrance carriers, ink jet stabilizers, and so on *(1–6)*.

The production of the CGTase enzyme is usually accompanied by other amylases that reduce CD yield because they increase the contents of glucose and maltose in the reaction medium and these saccharides inhibit the enzyme *(7,8)*. Therefore, purification of the enzyme is beneficial for higher CD production, and a highly efficient technique for that is biospecific affinity chromatography using β-CD as the ligand *(9,10)*.

Immobilization of enzymes has been applied successfully for creating economical continuous industrial processes *(11,12)*, and the conjunction of the fluidized-bed reactor (FBR) with immobilized enzyme technology still may lead to potential increased benefits, such as smaller reactors, shorter reaction time, and greater enzyme productivity (kilograms of product/kilogram of enzyme) *(13)*.

The objective of this study was to purify CGTase by biospecific affinity chromatography and immobilize it into controlled pore silica (CPS) particles, and then to characterize kinetically the immobilized enzyme, apply it in a fluidized-bed continuous reactor process for producing CDs, and compare the results with conventional liquid-phase technology. This enzyme, originally from an alkalophillic *Bacillus* (strain 1.1), was cloned in *Escherichia coli* by Schmid et al. *(14)*.

Materials and Methods

Substrate

The substrate was dextrin 10 supplied by Fluka Chemie AG (Buchs, Switzerland), article 31410. Its average mol wt was 1672 Daltons.

Enzyme

The enzyme CGTase (EC2.4.1.19) was obtained as a lyophilized powder, containing 193.1 mg of protein/g of powder. It was a gift from Wacker (Consortium für Elektrochemishe Industrie GmbH, Munich, Germany). The mol wt of this CGTase is 75,000 Daltons *(10)*. One unit of enzyme activity corresponds to the quantity of enzyme that produces 1 μmol of β-CD/min under specified conditions. This CGTase had a specific activity of 63.1 U/mg of protein, at 50°C, pH 8.0, and 5 mg/mL of dextrin 10.

Supports

Sepharose 6B, acquired from Sigma (St. Louis, MO), was used as support for biospecific affinity chromatography purification of CGTase. CPS

was the support for immobilizing the CGTase. It was a gift from Sucrerie Vanciennes (France), and the CPS particles had an average diameter of 0.42 mm, with a particle-size distribution in the range of 0.351–0.589 mm.

CGTase Activity

Free enzyme activity was measured by reacting a 3-mL mixture of diluted enzyme and substrate, containing 1.23×10^{-4} mg/mL of CGTase; 5 mg/mL of dextrin 10; 0.01 *M* Tris-HCl buffer, pH 8.0; and 5 m*M* $CaCl_2$. For each enzyme sample, seven test tubes were used, and these were collected at 5-min intervals for 30 min. The reaction was stopped by boiling the mixture for 5 min, and the solution was stocked in a refrigerator for later assay of β- and γ-CDs. The concentrations of β- and γ-CD as a function of time were adjusted by straight lines. The angular coefficient of these lines yields the enzyme activity for producing β- and γ-CD, respectively. Several enzyme concentrations were tried, and for 1.23×10^{-4} mg/mL, i.e. 1.64×10^{-9} *M*, a perfect straight line was obtained.

The immobilized CGTase activity was measured in a 100-mL batch microreactor, containing a stainless steel basket in which the immobilized enzyme particles were retained. Intense agitation was provided by a magnetic bar to guarantee good liquid-particle mass transfer. The immobilized enzyme (IE) sample (0.5 g) was taken from IE dried in a vacuum filter. A similar sample was used for determination of the IE humidity in an oven at 105°C. The liquid solution bathing the IE in the activity test had the same composition as for the determination of the activity of the free enzyme. One-milliliter samples were taken from the microreactor mixed with 1 mL of distilled water at 100°C and boiled for 5 min. These samples were collected at 2- or 5-min intervals for up to 30 min, the shorter interval being used with more active IE. After 30 min, the IE basket was removed from the microreactor and three more samples were taken up to 45 min, to detect the presence of any leached enzyme in the solution. The samples from this test were stocked and assayed as for the free enzyme activity test.

Purification of CGTase

The CGTase enzyme was purified by biospecific affinity chromatography with an adaptation of the method used by Berna et al. *(10)*. The chromatographic support was derivatized by activation with bisoxirane and coupling with the biospecific ligand β-CD following the steps depicted in Fig. 1.

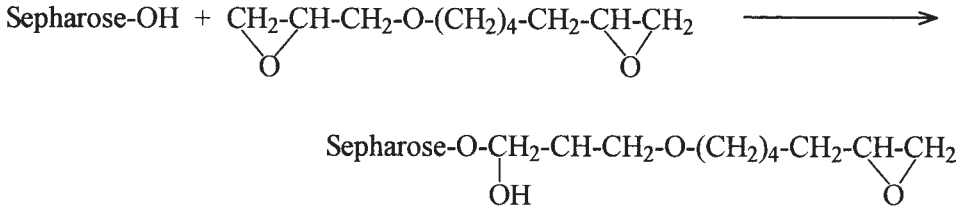
CGTase was purified by using 20 mL of the derivatized gel in a chromatographic column that was jacketed with water at 4°C. Chromatographic buffer (30 m*M* Tris-HCl, pH 8.0, and 20 m*M* $CaCl_2$) was supplied as downflow using a postcolumn peristaltic pump, with a flow rate of 0.4 mL/min. After column stabilization, 3.48 g of enzyme powder dissolved in 50 mL of the same buffer was applied at the top of the column, and impurities were washed out with 750 mL of buffer. CGTase was then recov-

Step 1: Activation of Sepharose with bisoxirane (1,4 butanediol diglycidyl ether)

Reagents: 1 mL of bisoxirane for each g of vacuum-dried Sepharose 6B, plus 1 mL of NaOH 0.6 *M* containing 2 mg of $NaBH_4$

Reaction conditions: 25°C, 8 h.

Washing: After activation the gel is thoroughly washed with d.w. and stabilized for 5 min with 5 mL NaOH 0.1 *M*.



Step 2: Coupling of the biospecific ligand β-cyclodextrin (β-CD)

Reagents: 2 mL of NaOH 0.1 *M* containing 20% w/v of β-CD for each g of vacuum-dried activated Sepharose 6B

Reaction conditions: 45°C, 16 h.

Washing: After coupling the gel is thoroughly washed with d.w. and then with buffer (Tris-HCl, 50 m*M*, pH 8.0).

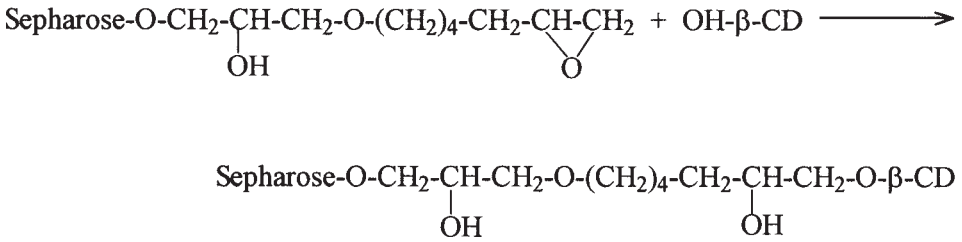


Fig. 1. Preparation scheme for the production of the biospecific affinity chromatographic support. d.w., distilled water.

ered by elution with 250 mL of buffer containing 10 mg/mL of β-CD. Samples of 2.5 mL were collected and analyzed for β-CD, CGTase activity, and protein.

Immobilization of Purified CGTase

Purified CGTase recovered from the affinity chromatography procedure was immobilized into CPS by the method of covalent bonding *(15)* as adapted by Catry and de Moraes *(16)*. CPS was hydrated with nitric acid (1:20) for 1 h at 82°C, and then silanized with 0.5% (v/v) γ-aminopropyltriethoxysilane, pH 3.0–4.0, for 3 h at 75°C.

After elimination of the excess silane and drying at 105°C for 15 h, the support was activated with 2.5% (v/v) glutaraldehyde, buffered at pH 7.0 with phosphate buffer, for 1 h at 26°C. Finally, purified CGTase was immobilized by contacting the solution eluted from the affinity column with the washed and dried activated CPS. The reaction was maintained for 24 h at 26°C.

Fluidized-Bed Expansion as a Function of Liquid Velocity

Bed expansion experiments were conducted in the FBR to determine the relation between bed porosity (ε) and fluidizing-fluid superficial velocity (u). The Richardson and Zaki *(17)* correlation for bed expansion, Eq. 1, was adjusted to the data, and in conjunction with Eq. 2 it yielded these parameters: bed expansion coefficient (n) and the free-falling particle terminal velocity (u_t).

$$(u/u_{tc}) = \varepsilon^n \tag{1}$$

$$\log u_{tc} = \log u_t - d_p/d_i \tag{2}$$

in which u_{tc} is the particle terminal velocity corrected by the tube wall effect *(16)*; d_i is the fluidized-bed column internal diameter; and d_p is the particle mean diameter. The bed porosity (ε) is calculated with Eq. 3:

$$\varepsilon = 1 - M_s/(\rho_p \cdot A_R \cdot H) \tag{3}$$

in which A_R is the cross-sectional area of the column; H is the fluidized-bed height; M_s is the mass of solid particles loaded into the column; and ρ_p is the particle density.

For these experiments, distilled water at room temperature was used as the fluidizing fluid, because the substrate solution at the FBR conditions (10% [w/v] dextrin at 50°C) has a density (1.022 g/cm^3) and viscosity (0.009 *P*) sufficiently close to that of distilled water at room temperature (0.998 g/cm^3 and 0.01 *P*, respectively) at 22°C. In addition to economizing the substrate, the use of water also facilitates the experiment because there is no need to apply temperature control.

A sample of 25 g of CPS particles was first submitted to vacuum to remove the air contained within the pores. Then water was added under vacuum, completely covering the solid, and the thoroughly wetted particles were loaded into the fluidization column. Afterward, liquid flow was set on and the expanded bed adjusted to its maximum allowable height (96 cm), avoiding any loss of particles from the column. Next, the flow rate was decreased slowly in steps and maintained for 20 min in each condition to permit the establishment of steady state, and the bed height and flow rate were registered. This continued until the bed height was allowed to shorten uniformly to the settled-bed height.

Production of CDs with Immobilized CGTase

The conversion of the substrate to cyclodextrins as a function of the residence time in an FBR of immobilized CGTase was measured at 50°C,

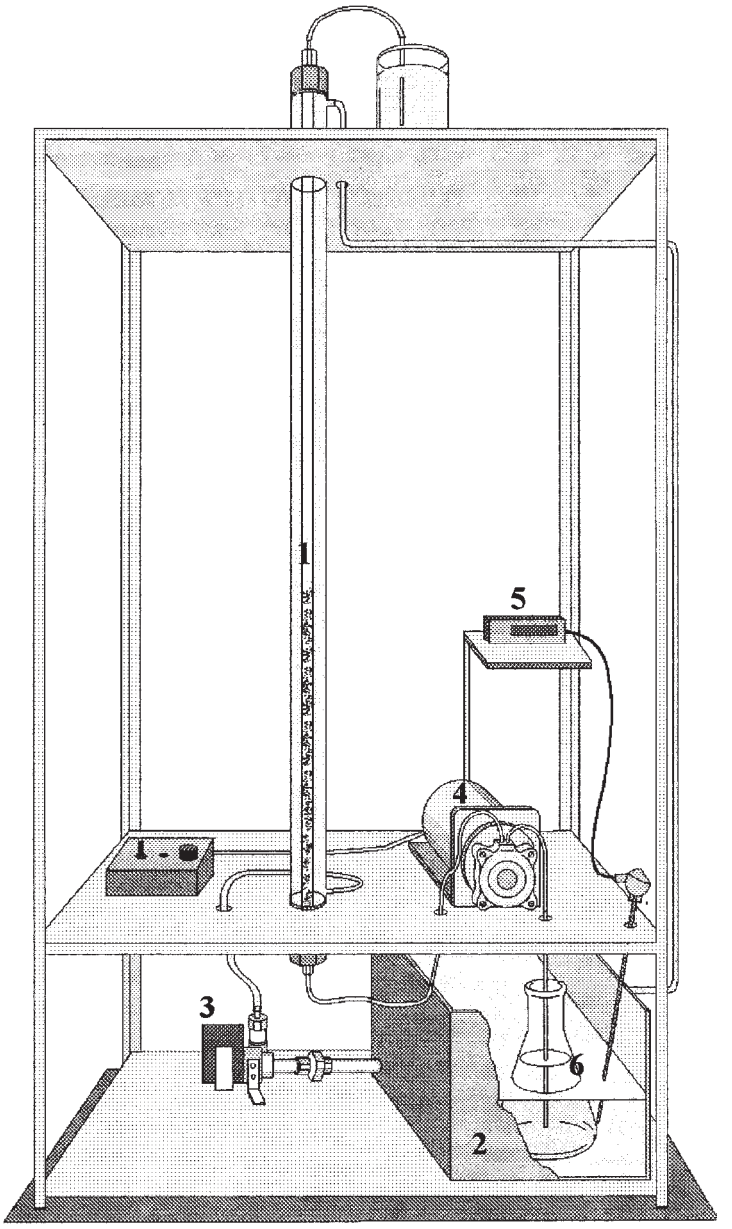


Fig. 2. Experimental setup of FBR, used for CD production: 1, jacketed glass column; 2, water reservoir used for thermocontrol of the reactor and feed solution; 3, pump used for water recirculation; 4, peristaltic pump used for feeding the substrate; 5, element for temperature control; 6, substrate reservoir.

pH 8.0, nominal bed porosity of $\varepsilon = 0.5$, and superficial liquid velocity of $u = 3.74$ cm/s. These fluidization conditions were chosen because early work with immobilized glucoamylase has shown that lower bed expansion leads to higher conversions *(13)*. The substrate solution contained 100 g/L

of dextrin 10, Tris-HCl buffer (10 m*M*), and $CaCl_2$ (5 m*M*). It was injected at the bottom of the reactor column (*see* Fig. 2), setting an upward flow of 7.5 mL/min. After flow and temperature conditions were stabilized, 5-g aliquots of immobilized CGTase were loaded into the reactor. For each aliquot a period of 30 min was allowed so that steady-state conditions were reestablished. Then the height of the fluidized bed of particles was measured, and 10-mL samples of the effluent were collected at 10-min intervals for 1 h. Next, liquid flow was switched off, the bed was gently tapped, and the bed height was measured. Immediately afterward another aliquot of IE was charged into the column and the aforementioned procedure was repeated until the maximum allowed fluidized-bed height (96 cm) was reached. The effluent samples were stocked at 4°C for later assay of β- and γ-CD concentrations.

Assay Methods

The concentration of β-CD was measured at 550 nm by the dye-extinction method using phenolphthalein, as developed by Vikmom *(18)* and modified by Hamon and de Moraes *(19)*. For γ-CD assay, complexation with bromocresol green increases the dye absorption at 620 nm, according to the method of Kato and Horikoshi *(20)*, and the modification introduced by Hamon and Moraes de *(19)* was used. Protein concentration was determined by the method of Bradford *(21)*, using bovine serum albumin as standard protein.

Results and Discussion

Purification of CGTase by Biospecific Affinity Chromatography

Berna et al. *(10)* purified the same CGTase enzyme, available as a liquid solution containing 1.4 mg/mL, and have shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis that the biospecific affinity chromatography applied in the present study produces a homogeneously pure CGTase. We also found that after the washing step that removes the contaminating proteins (34.7% of total protein recovered) (Table 1) from the column, the elution with chromatographic buffer that follows did not show any protein or CGTase activity for the first 20 mL eluted (Fig. 3). Additionally, when protein started to elute from the column, Fig. 3 shows that the profiles for enzyme activity and protein concentration, as a function of the eluted volume, coincide. CGTase recovered from the column corresponded to 65.3% of total protein recovered. Total protein recovered amounted to 94% of the protein applied to the column (Table 1). The purified enzymatic solution had a protein concentration of 1.64 mg/mL and a specific activity of 73.8 U/mg of protein at 50°C, pH 8.0, whereas the crude enzyme had a specific activity of 63.1 U/mg of protein at the same conditions. Hence, the purification factor, defined as the ratio of the specific activity after purification and the activity of the crude enzyme, was 1.2, and the purification yield, defined as the percentage of the total activity recovered, was 72%

Table 1
Purification of CGTase from Alkalophilic *Bacillus* Cloned in *E. coli*, by Biospecific Affinity Chromatography, Using β-CD as Ligand

Enzyme	Specific activity (U/mg)	Total protein (g)	Protein relative to stock protein (%)	Protein relative to total recovered protein (%)	Total activity (10^3 U)	Purification yield (% of activity recovered)	Purification factor (ratio of specific activities)
Stock	63.1	2.67	100	—	168.3	100	1.0
Purified	73.8	1.64	94	65.3	120.8	72	1.2
Impurities	0	0.87		34.7	0	—	—

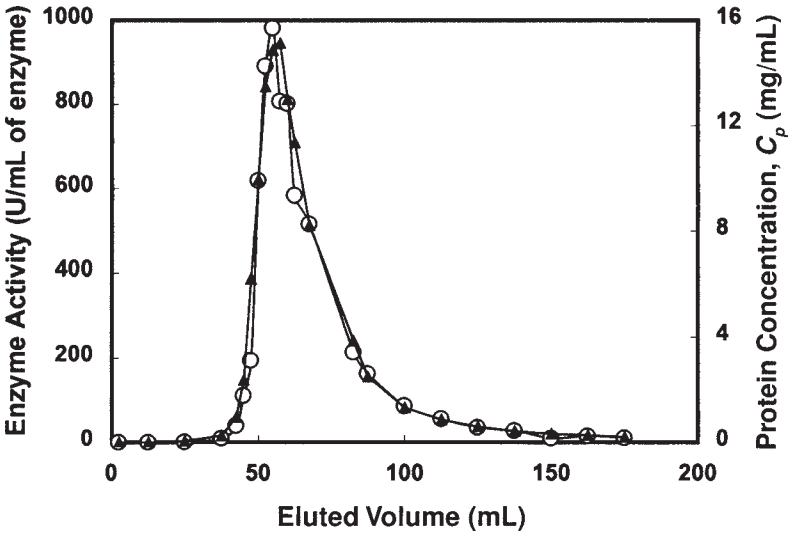


Fig. 3. Profiles for protein concentration and enzyme activity as a function of the eluted volume. Elution buffer: 30 m*M* Tris-HCl buffer, pH 8.0; 20 m*M* $CaCl_2$, and 10 mg/mL of β-CD. Flow rate: 0.4 mL/min. (○) Activity; (▲) protein.

(Table 1). Berna et al. *(10)* obtained a purification factor of 1.4 and a purification yield of 79%, and recovered 87.7% of their initial protein. The fact that the purification factor was low in our work and in Berna et al.'s *(10)* work is consistent with the fact that the CGTase enzyme was produced from a recombinant bacteria that gave, in the cell-free supernatant of the fermentation medium, a very high proportion of CGTase protein. This proportion calculated from the CGTase recovered from the biospecific affinity purification is 61.4% in the case of the present study and 56.5% in the case of Berna et al.'s *(10)* study.

Comparing the specific activities at 50°C and pH 8.0 of the purified CGTase for producing β- and γ-CD, it was observed that for β-CD the activity was 73.8 U/mg and for γ-CD it was 24.9 μmol of γ-CD/(min·mg of protein). These results give a ratio of 3 for β-CD:γ-CD initial production ratio, and consequently this enzyme is classified as a β-CGTase.

Immobilization of Purified CGTase

CGTase immobilization fixed 4.7 mg of protein (which was the affinity purified CGTase enzyme)/g of dried CPS support. This represents a protein fixation yield of 28.9%. de Moraes et al. *(22)* observed a protein fixation yield of 30% and 10.7 mg of protein/g of dried CPS support. However, the CGTase enzyme used by de Moraes et al. *(22)* was not purified, and it is likely that smaller proteins that diffuse faster than CGTase would have been immobilized to the detriment of CGTase immobilization. This hypothesis is consistent with the low fixed activity obtained by these investigators (9%), much lower than their protein fixation yield (30%). By contrast, in the present study, fixed activity was 28.7%, as shown in Table 2,

Table 2
Total Activity of Enzymatic Solution, Activity of IE, and Activity Yield

Enzymatic solution			IE[a]		
Total activity (μmol β-CD/min)					
Before immobilization	After immobilization	Fixed activity (%)	Maximal theoretical activity (U/g_{IE})	Experimental activity (U/g_{IE})	Activity yield (%)
115×10^3	82.0×10^3	28.7	344	8.6	2.5

[a]Ninety-six g_{IE} was produced.

hence very close to the protein fixation yield (28.9%). The proximity of these two later results confirms the purity of the CGTase used for immobilization in the present study.

Crump and Rozzell *(23)* immobilized the CGTase from *Bacillus macerans* also in silica particles and by the same covalent attachment method. However, they fixed 30–50 mg of protein/g of support, a quantity much larger than obtained in the present study, or in de Moraes et al.'s *(22)* work.

Specific Activity of Immobilized CGTase

The following specific activities resulted for the CGTase forms used in this study: enzyme free in solution, nonpurified and purified, 63.1 and 73.8 U/mg of protein, respectively. The immobilized CGTases gave a specific activity of 1.8 U/mg of protein. Therefore, on immobilization CGTase activity was reduced by about 40 times. A high reduction in activity was also observed by de Moraes et al. *(22)*. Reduction in specific enzyme activity on immobilization is usually attributed to steric hindrances within the particle pores, diffusional limitations, and modifications to the enzyme structure and/or active site. Note that because CDs are large circular molecules, they contribute to diffusional limitations and steric hindrances. No leaching of enzyme from the immobilized CGTase was observed during the activity determination tests.

Fluidized-Bed Expansion as a Function of Liquid Velocity

Fluidized-bed height (H) data as a function of liquid superficial velocity (u) were used in Eq. 3, with particle density $\rho_p = 0.9299$ g/cm^3 and column cross-sectional area $A_R = 2.00$ cm^2, to calculate the bed porosity (ε). Figure 4 shows the results. Equation 1 was adjusted to the data giving:

$$\text{for } 0.47 \leq \varepsilon \leq 0.7 \qquad \log u = 0.720 + 6.30 \log \varepsilon \tag{4}$$

$$\text{for } \varepsilon > 0.7 \qquad \log u = 0.344 + 3.98 \log \varepsilon \tag{5}$$

Therefore, in the log-log plot of Fig. 4, a change of inclination is observed for the adjusted straight lines as porosity changes from low to high values. This phenomenon was already observed with other particles by Riba and Courdec *(24)*.

Equation 5 was used with Eq. 1 to determine the bed expansion coefficient (n), and with Eq. 2 and particle mean diameter $d_p = 0.42$ mm, column diameter $d_i = 15.97$ mm to yield the particle free-falling terminal velocity (u_t) (Table 3). The minimum fluidization velocity (u_{mf}) is obtained at the intersection of the straight line given by Eq. 4 with $\varepsilon = 0.47$, the bed porosity observed at the minimum fluidization condition.

Table 3 presents the experimental results obtained for n, u_t, and u_{mf} along with theoretical values calculated by the correlation given by Richardson and Zaki *(17)* and Kunii and Levenspiel *(25)*. Agreement is good for n and u_{mf}, but for u_t the discrepancy is high (35.7%). High deviation for the fluidization parameters is not uncommon *(17)*. In the case of the data

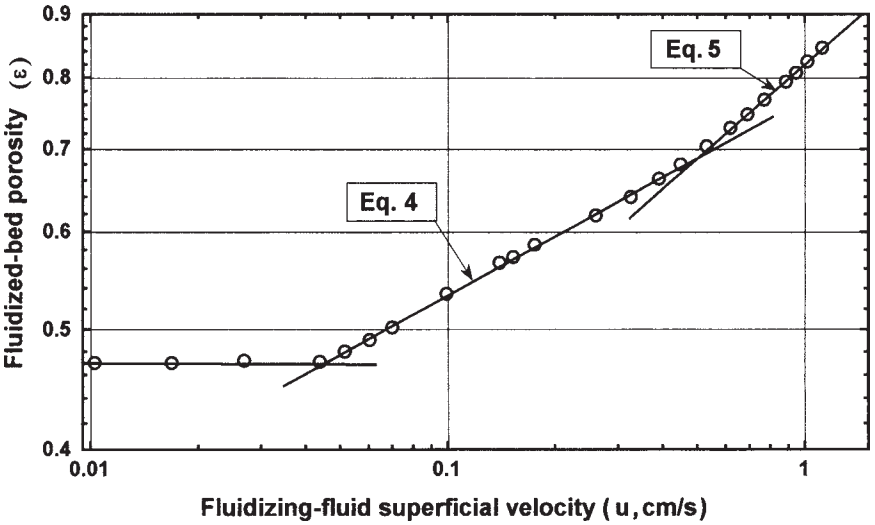


Fig. 4. Fluidized-bed porosity (ε) as a function of superficial liquid velocity (u).

Table 3
Comparison Among Experimental Values Obtained for Fluidization Parameters[a]

Parameter	Experimental value	Theoretical value	Difference (%)
u_t (cm/s)	2.350	3.180	35.7
u_{mf} (cm/s)	0.045	0.051	13.3
n	3.980	3.920	1.5

[a] u_t (particle free-falling terminal velocity); u_{mf} (minimum fluidization velocity); and n (bed expansion coefficient).

of Fig. 4, the discrepancy might originate from the transition observed in the inclination of Eqs. 4 and 5. Perhaps at still higher bed porosity above ε = 0.85, the inclination of the curve in Fig. 4 would conform to the theoretical value.

Production of CDs with Immobilized CGTase

Figure 5 shows the data obtained for settled bed height (H_0), fluidized-bed height (H), fluidized-bed porosity (ε), and effective residence time (t_{ef}) as a function of IE mass (M_s) charged into the FBR. The effective residence time (t_{ef}) was calculated by Eq. 6:

$$t_{ef} = V_l/v = (HA_R\varepsilon)/(uA_R) = H\varepsilon/u \quad (6)$$

in which v is the liquid flow rate, and V_l is the interstitial volume of liquid in the particle bed.

It can be observed in Fig. 5 that bed porosity (ε) was practically constant, and that H_0, H, and t_{ef} were linear functions of M_s, as expected, since the same flow rate was used for all the data in Fig. 5. These results confirm

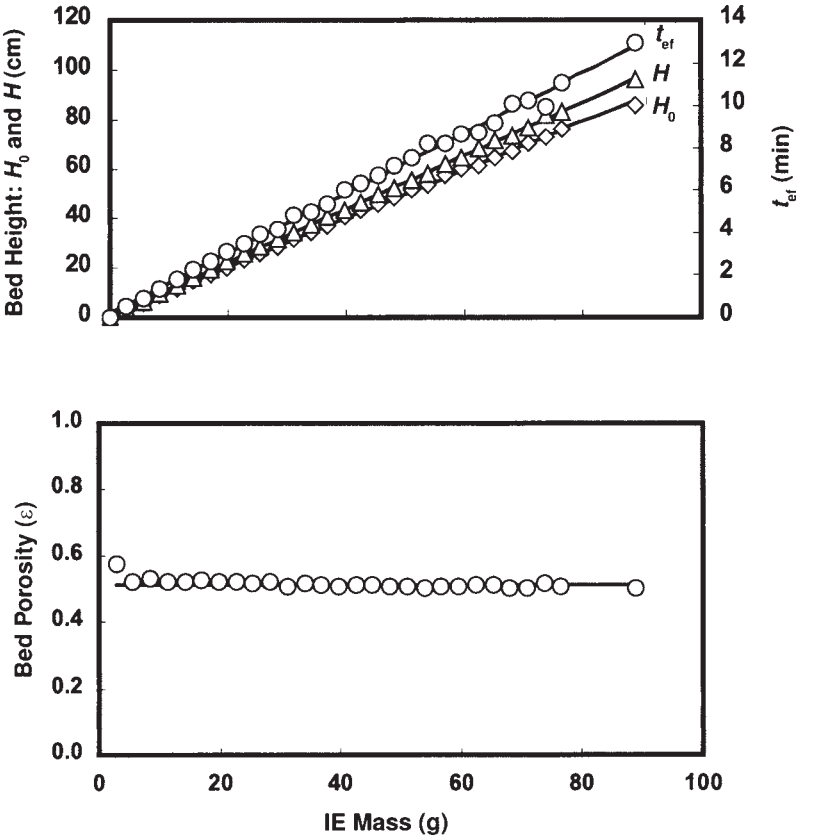


Fig. 5. Settled-bed height (H_0), fluidized-bed height (H), bed porosity (ε), and effective residence time (t_{ef}) of the fluidizing fluid, as a function of IE mass (M_s) loaded into the FBR. The average superficial fluid velocity was 3.79 cm/s.

what was planned, i.e., to vary bed height by varying IE load but keeping the fluidization conditions constant.

Figure 6 shows β- and γ-CD concentrations at the FBR outlet as a function of the IE mass loaded into the reactor. Figure 7 presents the conversion of the substrate to β-, γ-, and total CD. These conversions in percentage were calculated by Eqs. 7, 8, and 9, respectively.

$$X_{A,\beta\text{-CD}} = 100C_{\beta\text{-CD}}/C_{A0} \tag{7}$$

$$X_{A,\gamma\text{-CD}} = 100C_{\gamma\text{CD}}/C_{A0} \tag{8}$$

$$X_{A,\text{total}} = X_{A,\beta\text{-CD}} + X_{A,\gamma\text{-CD}} \tag{9}$$

in which C_{A0} is the true value of the initial concentration of the substrate discounting humidity (96.7 g/L); $C_{\beta\text{-CD}}$ and $C_{\gamma\text{-CD}}$ are the concentration of β- and γ-CD (g/L), respectively; and $X_{A,\beta\text{-CD}}$, $X_{A,\gamma\text{-CD}}$, $X_{A,\text{total}}$ are the conversion of the substrate to β-CD, to γ-CD, and the total conversion, respectively.

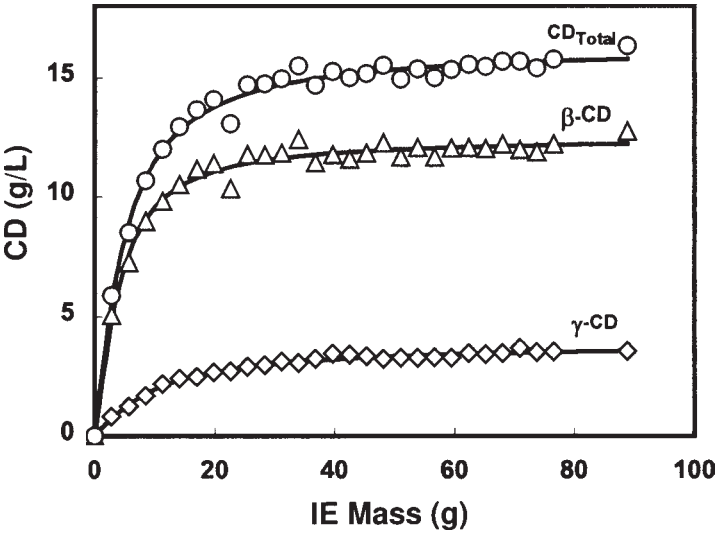


Fig. 6. Production of CD in grams/liter as a function of the IE loaded into the FBR. Conditions: 50°C, pH 8.0. The substrate solution was fed to the reactor with 100 g/L of dextrin 10, Tris-HCl buffer (0.01 *M*), and $CaCl_2$ (5 m*M*).

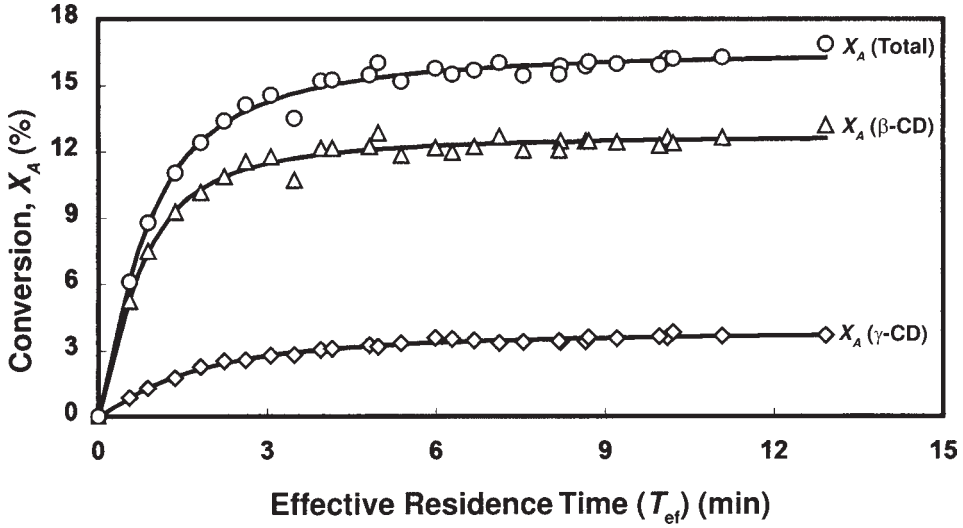


Fig. 7. Conversion of the substrate dextrin 10 to CDs as a function of the effective residence time (t_{ef}) of the fluidizing fluid. Conditions: 50°C, pH 8.0. The substrate solution was fed to the reactor with 100 g/L of dextrin 10, Tris-HCl buffer (0.01 *M*), and $CaCl_2$ (5 m*M*).

Maximum conversions achieved for an effective residence time, t_{ef}, of approx 13 min, which corresponds to an IE load in the reactor of 88.8 g (dry wt), or 417 mg of immobilized CGTase protein with a total enzymatic activity of 751 U, were $X_{A,}\beta_{\text{-CD}} = 13.2\%$, $X_{A,}\gamma_{\text{-CD}} = 3.7\%$, and $X_{A,\text{total}} = 16.9\%$.

CD concentrations were 12.76 and 3.57 g/L for β- and γ-CD, respectively. Reactor productivity under these conditions was 5.1 and 1.4 g of β- and γ-CD/(L·min), respectively.

Figures 6 and 7 show, as was observed for CD production by previous work *(3,8,16,19)*, that the conversion of the substrate to CDs is limited. This occurs because the reactions that produce CDs are reversible *(3,19)*. Additionally, these reactions are inhibited by the substrate *(3,8,19)* so that higher substrate concentration leads to lower conversion. The choice of initial substrate concentration of 10% (w/v) establishes a compromise between conversion and reactor yield and is sometimes used for the case of free enzyme technology *(19)*. For the IE case, the optimal initial substrate concentration is still to be determined, and it will depend on reactor productivity as a function of substrate concentration, as well as the costs of the substrate, product, and downstream separation.

The production of β-CD was 3.7 times that of γ-CD, confirming that the enzyme used also behaves as β-CGTase in the immobilized form. The molar selectivity for β-CD increased from 78%, a value determined by de Moraes et al. *(22)* for the free enzyme, to 80.3% for the immobilized CGTase of the present study, whereas for de Moraes et al. *(22)* it increased to 89%. In both cases, immobilization of the β-CGTase increased the selectivity for β-CD production.

Other investigators *(26–28)* have reached higher conversion of the substrate to CDs (46–70%). However, they have used usually much less concentrated substrate solutions (1% or less) and have also used longer reaction times (6–24 h).

As can be seen in Fig. 7, for an effective residence time of only 4 min, which corresponds to a load of 25.5 g_{IE} and an expanded bed height of 28.4 cm, a total conversion of 15.2% can be obtained. CD concentrations in this case were 11.8 and 2.9 g/L, for β- and γ-CD, respectively, leading to a higher selectivity for β-CD, namely 82%. It might be economically feasible to use this condition of t_{ef} equal to 4 min because in this case reactor productivity for β-CD increased 10 times, reaching 52 g of β-CD/(L·min). Reactor productivity for γ-CD increased 9.3 times, reaching 13 g of γ-CD/(L·min). The concentrations of β- and γ-CD in this case are equivalent to that obtained by Hamon and de Moraes *(19)* with a batch reactor and free enzyme. However, for Hamon and de Moraes *(19)*, these values were achieved after 24 h of reaction, and consequently, the batch reactor productivity was low: 0.008 g of β-CD/(L·min). The productivity for β-CD in the case of the FBR with an effective residence time of 4 min is then 6500 times higher than that obtained with free enzyme in a batch reactor.

Considering the high productivity achieved with the fluidized-bed technology using immobilized CGTase, which compares quite favorably with the batch reactor with free enzyme, it seems that the former technology has great potential for industrial application.

Conclusion

Immobilization of Wacker β-CGTase increased both the selectivity and reactor productivity for β-CD. In the case of 4 min of reaction time, production reached a selectivity of 82% for β-CD, with β- and γ-CD concentrations at 11.8 and 2.9 g/L, respectively. Reactor productivity was high: 52 and 13 g for β- and γ-CD/(L·min), respectively. These attractive results of the FBR with immobilized CGTase might lead to the development of an economical industrial process for producing CDs.

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