Hydrolysis of Starch with Immobilized Glucoamylase

A Comparison Between Two Types of Expanded-Bed Reactors

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Received June 5, 1989; Accepted September 13, 1989

ABSTRACT

Glucoamylase (E.C.3.2.1.3) covalently immobilized onto chitin particles (d_{st} = 0.37 mm) was examined in two types of continuous bench-scale reactors (180 mL) fed with hydrolyzed manioc starch (15%, w/v): a two-phase reactor (liquid expanded-bed) and a three-phase reactor (air expanded-bed). Several conditions of continuous operation were investigated, varying the biocatalyst load (16.7, 37.2, and 54 g/L) into the reactor and the hydraulic residence time. The best results were achieved with the two-phase reactor, which operated continuously for 20 d and showed a decrease of only 6% in conversion (starch to glucose). Conversion levels of 96% were obtained with a hydraulic residence time of about 4 h. A simple mathematical model was able to describe the experimental results of the two types of reactors considering biocatalyst deactivation.

Index Entries: Immobilized glucoamylase; starch hydrolysis; immobilized-enzyme bioreactors; enzymatic reactors.

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INTRODUCTION

One of the most important industrial applications of amylolytic enzymes in recent years has been the hydrolysis of starch raw materials to produce different concentration-grade glucose syrups. Among the many sources of starch raw materials available for hydrolysis, manioc or cassava, which is a native tropical plant, is recognized as an excellent starch source for ethanol production, mainly used in regions where sugar cane cannot be cultivated. Manioc is easily cultivated even in nonfertile soils and in climatic conditions adverse to many other plants.

The use of immobilized glucoamylase in the saccharification step of glucose-syrup production presents many advantages, as indicated by Linko et al. (*1*) and Nakhapetyan et al. (*2*). One of these is the reduction of residence time in the saccharification reactor, which is an important advantage when the process is oriented to ethanol production.

Glucoamylase has been covalently immobilized onto several different support materials through the formation of a Schiff base, as reported by Stanley et al. (*3*), Toldra et al. (*4*), Fiedureck et al. (*5*), and Lobarzewski et al. (*6*). In the work reported herein, glucoamylase was immobilized onto chitin particles. Chitin was chosen as the support for enzyme immobilization because in a previous study carried out in our laboratory, it showed very promising results when compared with other supports, such as sand, ceramics, Merkogel®, quartz, alumina, diatomite, and Chromosorb® (Bon et al. (*7*]). Chitin is a relatively inert support, resistant to the chemical action of acids and alkalis. To enhance its reactivity, chitin has been reacted with hexamethylenediamine before treatment with glutaraldehyde, as proposed by Bon et al. (*8*). Another important advantage of chitin in comparison with other supports is its low cost, since it is easily obtained from abundant seafood-industry wastes (crab and shrimp shells).

The design of an adequate immobilized-enzyme reactor is an important feature in improving the efficiency of industrial processes that utilize immobilized enzymes. Fixed-bed reactors tend to plug and cause an excessive pressure drop when using biocatalyst particles of small particle size that are friable and have the tendency to adhere to each other, especially when using raw starch material. These inherent problems can be overcome by using an expanded- and fluidized-bed-type reactor. Biocatalyst in these reactors may easily be replaced, if necessary, without interference with the reactor operation (Vallat and Monsan [*9*]).

Considering the properties of our biocatalyst and the advantages of expanded-bed reactors as described above, the study reported herein was designed to evaluate the performance of two types of expanded-bed reactors: a two-phase (liquid expanded-bed) and three-phase (air expanded-bed) reactor operated continuously at different loads of immobilized enzyme. However, the sludge-bed reactor (three-phase, i.e., air, biocatalyst, and substrate solution), is not usually utilized as a reactor for immobilized

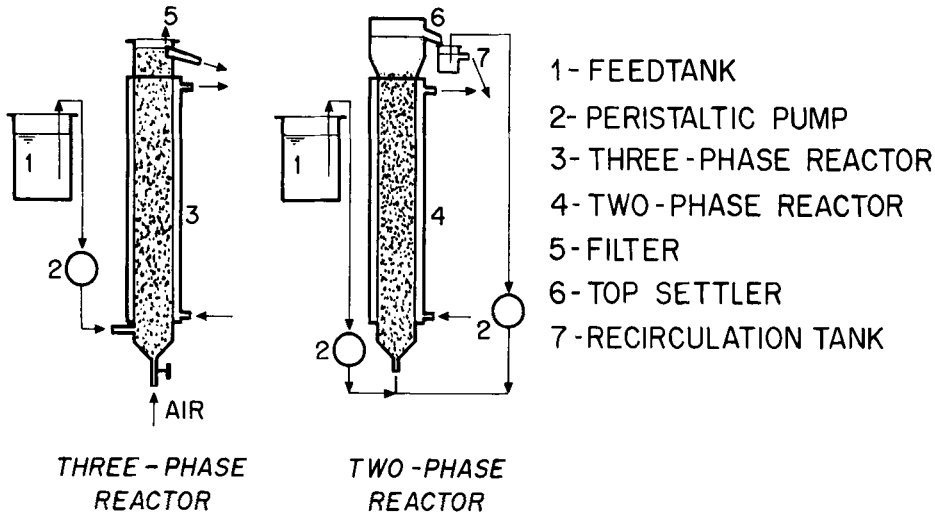


Fig. 1. Schematic diagram of the two-phase and three-phase reactors.

enzymes, for reasons such as the high cost associated with using air and the possible oxidation (and inactivation) of the enzyme itself. We have used this type of reactor in our studies to examine the stability of our biocatalyst preparation under conditions of higher agitation used to fluidize the bed, because, as was demonstrated in a previous study by Sant'Anna et al. (*10*), the immobilized enzyme showed good stability in experiments carried out with a pilot-scale three-phase reactor.

MATERIALS AND METHODS

Aspergillus niger glucoamylase (E.C.3.2.1.3.) from Novo Industri, Denmark (300 AGU/mL) was immobilized without purification onto chitin particles (Sauter diameter of 0.37 mm) activated with hexamethylene diamine and glutaraldehyde, as described by Bon et al. (*7*).

The substrates utilized were soluble starch (A. G. Reagen) and commercial manioc starch flour (Cassava do Brasil). The reactors were fed with a 15% (w/v) solution of hydrolyzed-manioc starch, which was previously prepared in a batch stirred reactor described by Freire (*11*).

The cooking and liquefaction of the manioc starch was performed in this reactor by the addition of α-amylase (E.C.3.2.1.1.), Termamyl type from Novo, in a ratio 0.1% (w/w) enzyme/manioc flour. The hydrolyzed manioc-starch solution that was produced (26-28 DE) was then cooled and its pH adjusted to 3.6-3.8, and it was stored until the continuous saccharification step.

The continuous saccharification reactors were glass columns with a nominal volume of 180 mL, as shown in Fig. 1. In the two-phase reactor

bed, expansion was promoted by the intensive recirculation of the effluent stream. The ratio between recirculation and feed flow rates varied from 11 to 560, depending on the hydraulic residence time and the biocatalyst load in the reactor. In the three-phase reactor bed, expansion was obtained by continuous air-flow injection from the reactor bottom. To avoid the loss of particles in the effluent stream, a top settler was installed in the two-phase reactor. A stainless-steel basket (150 mesh) was used as a filter in the exit tube of the three-phase reactor (*see* Fig.1).

Several experimental runs were carried out, varying the biocatalyst load (17, 37, and 54 g/L) and the nominal residence time (0.5–20 h) in both reactors, which operated at a constant temperature of 45°C. During these runs, the variables monitored were total reducing sugars (Nelson [*12*]) and glucose (GOD PAP-Merck Kit) concentration in the feed and exit streams; pH and temperature in the reactor; recirculation, feed, and air flow rates; loss of fine particles; and biocatalyst activity.

The immobilized-enzymatic activity was determined on samples (ca. 40 mg) withdrawn from the reactors and was performed in a batch basket reactor by measuring the initial rate of hydrolysis of a solution of soluble starch (5%, w/v). A unit of activity is defined as the amount of enzyme that catalyzes the liberation of reducing sugars equivalent to 1 μmol of glucose/min from the soluble starch solution at pH 4.4 and 45°C, as described by Freire.

RESULTS AND DISCUSSION

The experimental results concerning the continuous operation of the two-phase reactor with a nominal residence time of about 3.5 h are presented in Fig. 2 and show the conversion (starch into glucose) as a function of the operation time for three different immobilized-enzyme loadings (16.7, 37.2, and 54 g/L). The results demonstrated, as expected, that an increase in immobilized-enzyme load resulted in higher conversions. The results also demonstrated the stability of the immobilized enzyme under continuous operation conditions.

The increase in conversion levels associated with higher loads of immobilized enzyme and longer residence times is shown in Fig. 3 for the two-phase reactor (open symbols). The experimental data of Figs. 2 and 3 are the means of triplicate experimental runs under steady-state conditions. Under the maximum loading constraints (54 g/L) imposed by the configurational design of the two-phase reactor, the conversion of starch to glucose was 95.8 and 96.6% (DE), with a reactor hydraulic residence time of about 4 h (or a nominal residence time of 5.8 h). These results indicate that the saccharification reactors may be significantly smaller than that used in the conventional process for ethanol production with free enzyme.

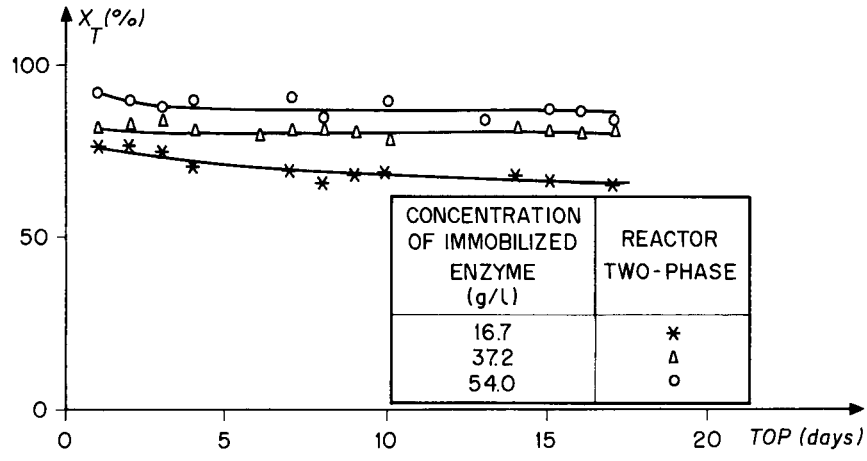


Fig. 2. Total conversion as a function of the operation time for three different immobilized-enzyme loadings in the two-phase reactor.

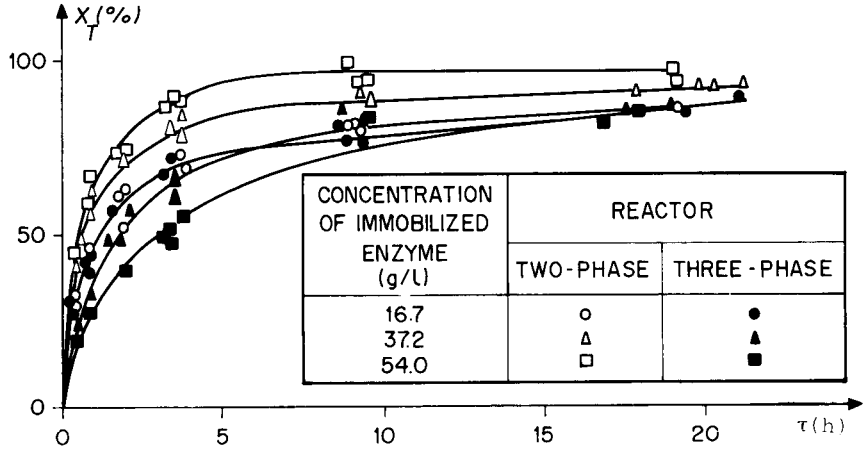


Fig. 3. Total conversion as a function of the nominal residence time for three different immobilized-enzyme loadings in both reactors.

The results shown in Fig. 4 for the three-phase reactor indicate that the conversion increase promoted by higher immobilized-enzyme loads was surpassed by the enzyme deactivation observed at the beginning of the continuous operation. The reason for the loss of enzymatic activity of the immobilized glucoamylase was the removal of the polymeric layer covering the chitin particles, which was caused by attrition as a result of agitation in the reactor. This polymeric material was collected on the filter (*see* Fig. 1 [5]) and was found to contain glucoamylase activity.

The effect of oxidation resulting from air pumped into the reactor (i.e., three-phase reactor) was examined as a possible cause of the enzyme inactivation. An experiment was performed in which two identical batch

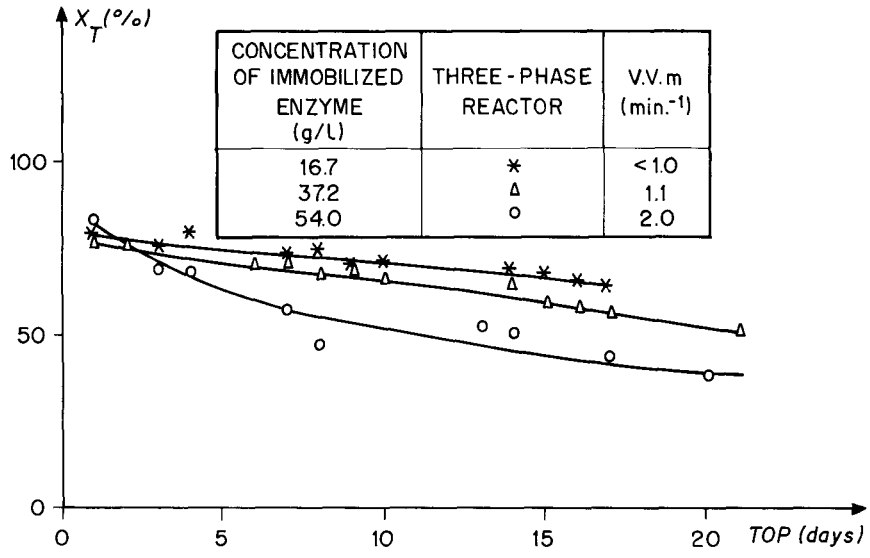


Fig. 4. Total conversion as a function of the operation time for three different immobilized-enzyme loadings in the three-phase reactor.

reactors operated with mild agitation, but only one of them received a constant air supply (2.0 vvm). After 20 d of continuous operation, the activity levels of the immobilized glucoamylase in the aerated and non-aerated batch reactors were almost the same indicating that air did not inactivate the immobilized enzyme.

To promote bed expansion at increased immobilized-enzyme loads, it is necessary to raise the rate of air flow to the reactor, in order to achieve the same bed expansion as occurs with a two-phase reactor. It therefore appears that the intensive mixing in the reactor, caused by air bubbles, resulted in a gradual stripping of the polymeric layer that contained immobilized glucoamylase, and that such losses were responsible for the reduced enzymic activity. As a consequence of the physical loss, the performance of the three-phase reactor was inferior at the two higher immobilized-enzyme loads (37.2 and 54.0 g/L) than that of the two-phase reactor (*see* Fig. 3). However, during the experiments with the minimum immobilized-enzyme load (16.7 g/L), the three-phase reactor was operated at the smaller specific flow rate, leading to a superficial air velocity close to the liquid superficial velocity in the two-phase reactor (0.45 cm/s). The results of the two reactors in terms of conversion (with respect to nominal residence time) were very similar (*see* Fig. 3). Thus, the deactivation of the immobilized enzyme diminished with the decrease of the specific air flow rate (vvm), so increasing the reactor volume (scale-up) should lower the vvm necessary for mixing, and we can expect that in a pilot or industrial three-phase reactor, the deactivation will be smaller than that observed in bench-scale reactors. However, the immobilized enzyme used in this

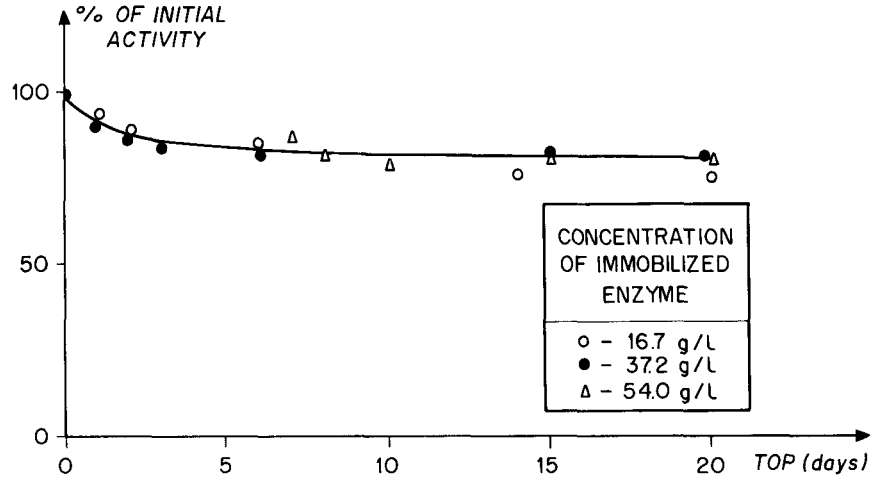


Fig. 5. Enzymatic activity decay in the two-phase reactor at three different immobilized-enzyme loadings.

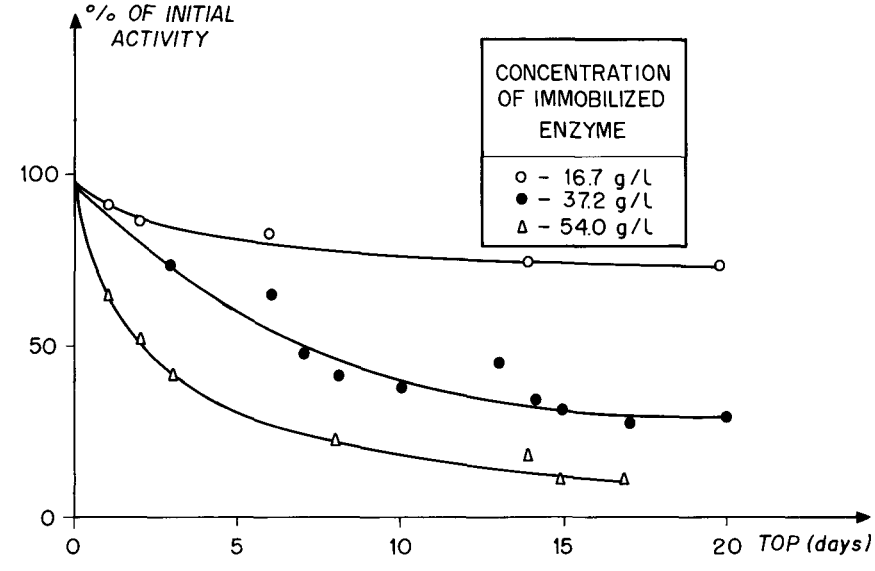


Fig. 6. Enzymatic activity decay in the three-phase reactor at three different immobilized-enzyme loadings.

work was found to be sensitive to mechanical stresses associated with intensive mixing.

The behavior of the enzymatic-activity decay was evaluated in both reactors at three different loadings, utilizing small samples of immobilized enzyme withdrawn from the reactors. The initial activity of the immobilized enzyme in all runs was about 1000 U/g of support. The decay of activity (expressed as a percentage of the initial value of enzyme activity) is presented in Figs. 5 and 6 for the two-phase and three-phase reactors,

respectively. The mathematical model of deactivation that best fits the experimental data obtained was the linear inverted model (Cardoso and Emery [*13*]). Using this model at the maximum immobilized-enzyme load (54 g/L), the following half-life times were obtained: 64(r=0.96) and 396 h (r=0.90) for the three-phase and two-phase reactors, respectively. The high estimated half-life under operational conditions for the two-phase reactor shows the excellent stability of the immobilized enzyme in this type of reactor. This value is one of the highest published in the literature (Stanley et al. [*3*]; Rugh et al. [*14*]; Cabral et al. [*15*]; Cabral [*16*]; and Lobarzwski et al. [*6*]).

The deactivation effects were different in each reactor, and also the decay of immobilized-enzyme activity was different at each load in the three-phase reactor (*see* Figs. 5 and 6) when the nominal residence time was varied. To take this into account, the parameter E_T/Q_a, utilized by Cabral (*16*) andd Allen et al. (*17*), is defined as follows:

$$E_T / Q_a = E_w \cdot C_B \cdot \tau$$

where

E_T = total enzymatic activity (units)
Q_a = fed flow rate (mL/min)
E_w = activity of the immobilized enzyme (U/g of support)
C_B = load of immobilized enzyme in the reactor (g/mL)
τ = nominal residence time (min) = reactor volume/fed flow rate (hydraulic residence time was defined in this work as the following ratio: liquid reactor volume/fed flow rate).

If the conversion in the saccharification step of both reactors is represented as a function of the parameter E_T/Q_a, as indicated in Fig. 7, only one curve represents the results obtained. The experimental data concerning the three-phase reactor is located in a region of the curve that corresponds to E_T/Q_a lower than 20.000, because in this type of reactor the enzyme deactivation was strong (lower E_T), so the maximum conversions were obtained only in the two-phase reactor (open symbols).

The maximum conversion for the three-phase reactor was 83.9% for an E_T/Q_a of about 17.000 U · min · mL^{-1}; in the case of the two-phase reactor, the conversion (starch to glucose in the saccharification step) was 92.0 or 95.8% (total conversion) for an E_T/Q_a of about 26.000 U · min · mL^{-1}.

The hyperbolic curve (Fig. 7) may be represented by the following equation:

$$X_R = 0.84\, E_T/Q_a / (940 + E_T/Q_a) \qquad (r=0.92)$$

The mathematical relationship reflects the "reactivity" of the immobilized enzyme support, and the displacement of the curve (Fig. 7) to the left side, which is of practical interest, may only be obtained with modifications in the support, on enzyme activity and the nature of the substrate.

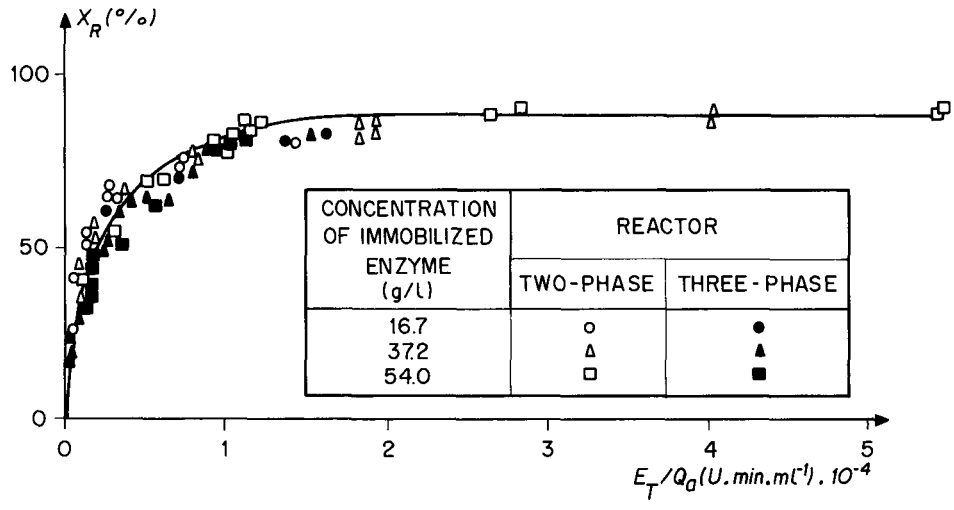


Fig. 7. Saccharification step conversion as a function of the parameter E_T/Q_a for three different immobilized-enzyme loadings in both reactors.

Assuming that for these reactors the liquid phase is completely mixed, and assuming first-order kinetics for substrate consumption, then it is possible to develop a simple mathematical model, as demonstrated in the Appendix.

However, to assume first-order kinetics it is necessary that $K_m >>> S$ (Michaelis model), but in this system the K_m for the immobilized enzyme was 7.8 g/L. This value is lower than the substrate concentration inside the reactor, even when conversion levels are high ($S = 8.0$ g/L). The assumption of a completely mixed reactor model may also be criticized, because tracer tests should be done to confirm it.

CONCLUSION

The use of glucoamylase immobilized onto chitin in a two-phase expanded-bed reactor should be a promising alternative for the saccharification step in the production of ethanol from raw starch materials. Maximum conversion was attained at reduced residence time, which leads to the size of reactors being considerably lower than the conventional process with free enzyme. The stability of the immobilized enzyme was excellent in this type of reactor, the estimated half-life under operational conditions was about 165 d.

The performance of the three-phase expanded-bed reactor was affected by the intensive mixing in this type of reactor. Deactivation of the immobilized enzyme was observed as a result of the mechanical removal of the polymeric layer covering the support.

The parameter E_T/Q_a takes into account the deactivation of the immobilized enzyme, so it is possible to describe all the experimental results of

continuous operation by a hyperbolic function, which may be used as a design equation.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to R. Dekker (CSIRO-Australia) for his valuable suggestions to improve this text. This work was supported by CNPq and Petrobras grants.

APPENDIX

Mathematical Model to Represent $X_R = X_R E_T/Q_a$

S = substrate concentration
G = glucose concentration

It is considered that

$$ds/dt = -f\,dG/dt$$

where f = yield constant

If a substrate first-order kinetics is assumed, then

$$r_s = dS/dt = -KES$$

where C_B = bioctalyst concentration (load) and E_w = biocatalyst activity.

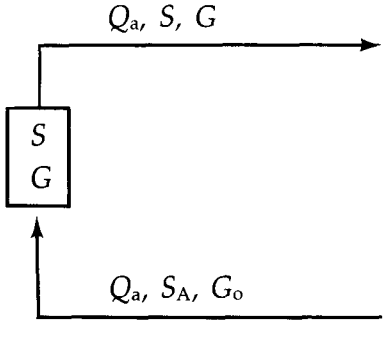
So,

$$r_s = -K\,C_B \cdot E_w \cdot S$$

and

$$r_G = -1/f\,r_s = K\,C_B \cdot E_w \cdot S/f \qquad (1)$$

If the liquid-phase mixing in the reactor is represented by a completely mixed model, then a glucose balance around the reactor in steady-state conditions leads to



$$Q_a \cdot G_o - Q_a\,G = -r_G \cdot V \qquad (2)$$

From Eqs. (1) and (2),

$$G - G_o = K\,\tau\,(C_B \cdot E_w/f) \cdot S \qquad (3)$$

as $S_A\ ^1\ S\ ^5\ f\ (G - G_o)$

$$S_A - S = f\,(G - G_o) \qquad (4)$$

$$\text{and } S_A = S_o - f\,G_o \qquad (5)$$

where S_o = substrate concentration of the original starch solution. Some glucose is produced in the previous treatment with α-amylase.

From Eqs. (5), (4), and (3) it results that

$$G - G_o = (K \tau C_B E_w / f)(S_o - fG) \tag{6}$$

and from the definition of conversion (X_R)

$$X_R = f[(G - G_o) / S_o] \tag{7}$$

If Eq. (7) is substituted into (6) and considering that

$$E_T / Q_a = \tau \cdot C_B \cdot E_w$$

the final result is:

$$X_R = [1 - f(G_o / S_o)] E_T/Q_a / (1 / K) + E_T/Q_a \tag{8}$$

Equation (8) is a mathematical relation that fits the experimental data of Fig. 7.

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