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A STUDY OF GELLED ENZYME ULTRAFILTRATION REACTORS

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Experiments were carried out in UF reactors with dynamically formed gelled enzyme artificial membranes. Unstirred batch systems and UF reactors with continuous recirculation of the substrate on the membrane were investigated. A significant increase in enzyme stability was tested in both systems. The enzymes used were acid phosphatase, urease, and β-glucosidase. The agreement between the experimental results and the predictions of a simple analytical model for the two classes of UF heterogeneous enzymatic reactors is generally satisfactory.

INTRODUCTION

There is increasing interest in heterogeneous enzymatic reactors containing immobilized enzyme to stabilize the enzymatic activity and to eliminate the requirement for recovery of enzyme. The scientific and industrial interest in this field is documented by the large number of publications which have appeared in the open literature (1–6).

Artificial polymeric membranes are used as immobilizing supports because of their relatively low cost and adaptability to a large number of enzymes. Problems such as (1) enzyme inhibition by-products, (2) enzyme separation from substrate and products, and (3) subsequent purification and application to continuous processes are easily solved when enzymes are immobilized on, or in, membranes. Membrane processes, including ultrafiltration and hyperfiltration, are accepted as unit operations. Moreover, the rapid development of new membrane types permits a rational selection of membrane support for a given enzyme–substrate combination. Recently, a study on the kinetic behavior of dynamically formed gelled enzyme ultrafiltration membranes was presented (7–8). Gelled enzyme membranes, involving labile immobilization at the membrane–solution interface, result from concentration polarization. These enzyme gelled

membranes are potentially useful in membrane separation processes when combination of selective mass transport across the membrane with a chemical reaction is desired. Simple analytical models are developed here to predict the kinetic behavior of two enzymatic reactors. The first type is based on a batch unstirred ultrafiltration process; the second is based on an ultrafiltration process with continuous recirculation of the substrate solution across the membrane.

For both systems the enzyme can be totally or partially immobilized in gel form on the pressurized face of the membrane, depending on the detailed fluid dynamics (7). The analytical results have been compared with experimental data. A satisfactory agreement between theory and experiment has been observed.

MATERIALS AND METHODS

Materials

The products used were acid phosphatase (E.C. 3.1.3.2) from Boehringen (Mannheim, West Germany) and sodium *p*-nitrophenylphosphate (BDH Italia, Milan) in 0.05 M citrate buffer, *p*H = 5.6, as a substrate; urease (E.C. 3.5.1.5) from Merck and urea in phosphate buffer, *p*H 7, as a substrate; β-glucosidase (E.C. 3.2.1.2.1) from Boehringer and *p*-nitrophenyl-β-D-glucopyranoside in acetate buffer, *p*H 5.2, as a substrate.

The amount of *p*-nitrophenol liberated by the enzymatic hydrolysis was read at 405 nm in a spectrophotometer. The amount of ammonia was read at 435 nm.

Unannealed asymmetrical cellulose acetate membranes characterized by 100% rejection for the enzymes and substantially no rejection for the substrates and products were used in all the experiments.

Methods

In the unstirred batch system, a fixed volume of enzyme solution is completely ultrafiltered through the semipermeable membrane, under an applied pressure of 2 atm. The UF cell is then connected to the pressurized reservoir containing the substrate solution. UF flow rate and the concentration of the reaction product in the permeate are measured at definite time intervals. A schematic flow sheet of the continuous ultrafiltration reactor is presented in Fig. 1. The experiments are carried out recirculating the substrate solutions, with an axial flow rate in the range 3×10^{-5} to 1.7×10^{-4} liters/sec. First the enzyme solution is ultrafiltered without recirculation, and subsequently the substrate solution, which is continuously

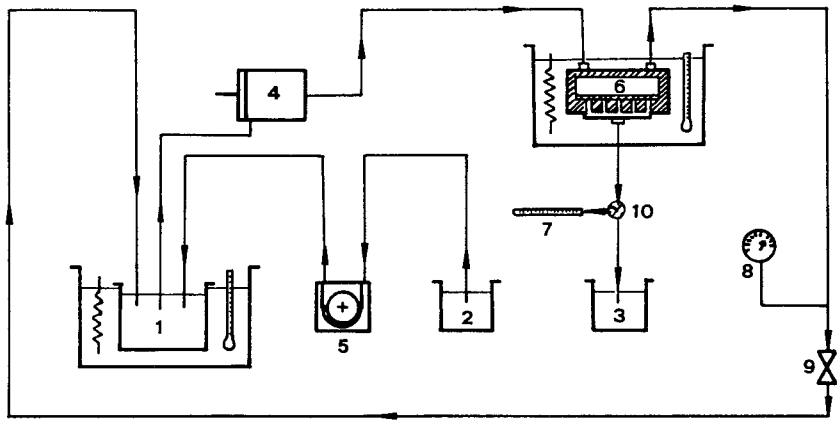

FIG. 1. Schematic of the recirculating ultrafiltration apparatus.

recirculated over the membrane is ultrafiltered. The total volume of the substrate solution in the reservoir (Vs) is kept constant by continuously adding buffer solution in an amount equal to the permeate flow rate, Q_T.

UNSTIRRED BATCH ULTRAFILTRATION REACTOR

The gelled enzyme membrane (see Fig. 2) is formed by ultrafiltering an aqueous enzyme solution through a membrane which is essentially permeable to the solvent and to the low molecular weight buffering solutes. The gel

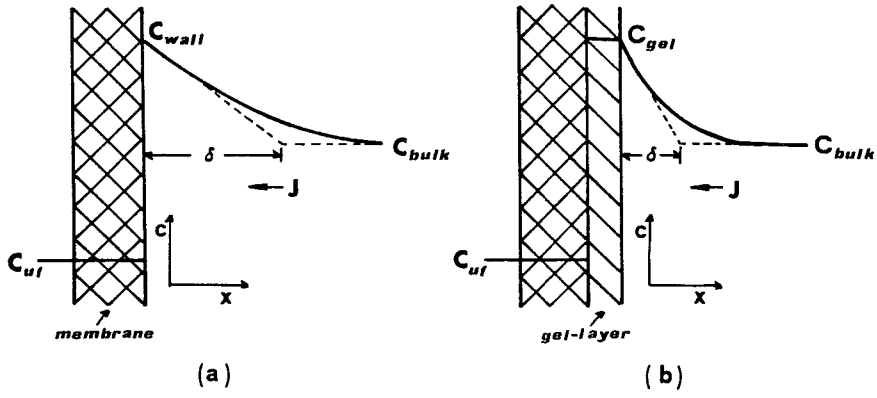

FIG. 2. Gel layer formation ultrafiltering a protein solution through a semipermeable membrane.

is completely stable over the time scale of these experiments. In this system the original ultrafiltration membrane acts only as a support for the gel layer and does not affect the activity of the gelled enzyme. Because of the negligible backdiffusion of the enzyme, the substrate concentration, C_s, is assumed to be constant with time in the reservoir. This assumption has been experimentally confirmed.

The analytical model is based on the following additional assumptions:

1. Plug flow occurs in the reactor.
2. The enzyme is completely gelled on the ultrafiltration membrane in an artificial layer of constant thickness, δ_E.
3. The substrate and concentration of products upstream and downstream from the composite membrane are everywhere uniform, assuming (a) zero membrane rejection for the substrate and for the reaction products and (b) negligible backdiffusion of enzyme from the gel (checked experimentally).
4. The reaction rate in the gel follows classical Michaelis–Menten equation, which is generally valid when the enzyme is in free solution:

$$R'_S = V'_{max} C_s / (K'_M + C_s) \tag{1}$$

where V'_{max} is the "apparent" maximum rate (when the substrate is no more rate controlling and K'_M is the "apparent" Michaelis constant. As commonly suggested, the word "apparent" shows that V'_{max} and K'_M do not have the same respective values as V_{max} and K_M observed for the enzyme in free solution.

A qualitative representation of the concentration profiles for substrate and products is presented in Fig. 3. A steady-state mass balance on the substrate, within the gel layer, may be written as

$$Q_T(dC_s/dx) = -\Omega R'_s \tag{2}$$

where Ω is the membrane surface, Q_T the volumetric flow rate, and x a coordinate perpendicular to the membrane surface.

By integration of equation 2, assuming the boundary

$$C_s = C_{s0} \qquad X = 0$$

we obtain

$$K'_M \log C/C_{s0} + C - C_{s0} = -\Omega k \tag{3}$$

From equation 3, for $x \geq \delta$, indicating with C'_s the values of the concen-

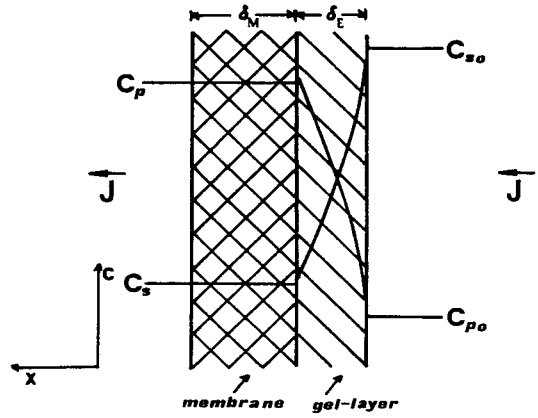

FIG. 3. Concentration profiles for substrates and products in the gel layer.

tration downstream the gel layer, we have

$$K'_M \ln(C'_s/C_{s0}) + C_{s0} - C'_s = V'_{\max}\,\Omega\delta_E/Q_T \tag{4}$$

The enzyme gel volume, V_E, must be directly proportional to the quantity of enzyme stored, by ultrafiltering, on the membrane surface, P_E; therefore,

$$V_E = \Omega\delta_E = P_E/\gamma_E\varepsilon_E \tag{5}$$

where γ_E is the specific gravity and ε_E the porosity of the enzyme in gel form. Introducing the degree of conversion, $\bar{X}$, as

$$\bar{X} = (C_{s0} - C'_s)/C_{s0} \tag{6}$$

one obtains

$$-K'_M \ln(1-\bar{X}) + C_{s0}\bar{X} = \nu P_E/Q_T \tag{7}$$

where ν is a constant given by the following expression:

$$\nu = V'_{\max}/\gamma_E\varepsilon_E \tag{8}$$

$V'_{\max}$ has been assumed to be constant because the enzyme concentration in the gel is constant; therefore, the concentration of active sites may also be assumed as constant. Equation 7 can be used to estimate the Michaelis constant K'_M by plotting $C_{s0}\bar{X}$ as a function of $\ln(1-\bar{X})$ and recognizing that the slope of the resulting straight line is equal to K'_M. The numerical value of the other parameter, ν, can be determined by extrapolating the experimental data for $\ln(1-\bar{X}) \to 0$ whereby $C_{s0}\bar{X} \to C_{s0} \to P_E/Q_T$.

The validity of this model has been tested by comparing different sets of experimental data with the analytical predictions. The degree of conversion is plotted as a function of substrate concentration in Fig. 4 for experiments carried out with different enzyme amounts gelled on ultrafiltration membrane. The experimental data were obtained in a discontinuous ultrafiltration reactor previously described (7,8). The enzyme was acid phosphatase (E.C. 3.1.3.2) and the experiments were carried out at constant temperature and constant transmembrane pressure drop.

The experimental behavior relating the degree of conversion with effluent flow rate is compared with the analytical predictions in Fig. 5.

Experimental results obtained with urease and β-glucosidase are presented in plots of degree of conversion as a function of substrate concentration in Figs. 6 and 7, respectively. The maximum deviation between theory and experiment is 10%. The agreement between the experimental data and the analytical predictions is therefore quite satisfactory.

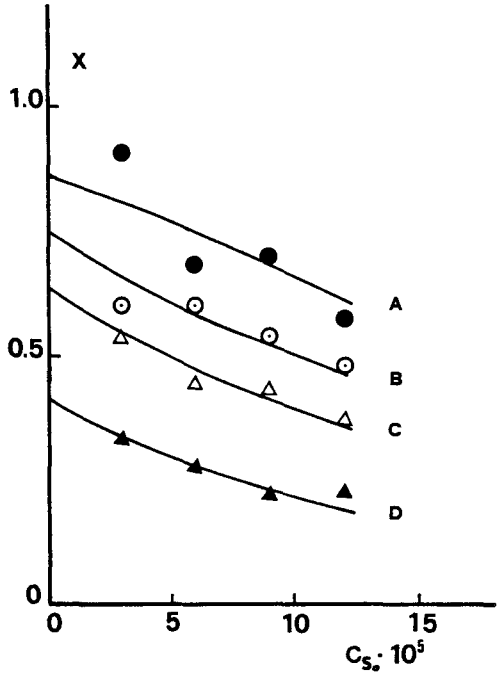

FIG. 4. Degree of conversion as a function of initial substrate concentration, in experiments carried out with different enzyme amounts (A, 6.4×10^{-6} g/cm^2; B, 5.1×10^{-6} g/cm^2; C, 4.0×10^{-6} g/cm^2; D, 2.6×10^{-6} g/cm^2) in an unstirred batch system. Enzyme, acid phosphatase; $\Delta P = 1.5$ atm; $T = 30$°C; full line, analytical prediction.

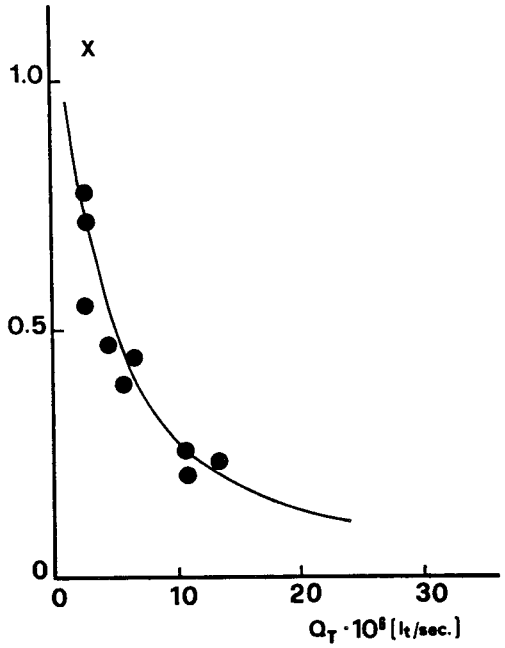

FIG. 5. Degree of conversion with the effluent flow rate. Full line, analytical prediction; enzyme, acid phosphatase (4×10^{-6} g/cm^2); $C_{s0} = 60$ M; $T = 30$°C.

ULTRAFILTRATION REACTOR WITH CONTINUOUS RECIRCULATION OF THE SUBSTRATE ON THE MEMBRANE

In the continuous recirculation process the substrate concentration in the reservoir cannot be assumed with time t because the reaction also takes place in the reservoir (see assumption 2, below). The analytical model developed for describing this process is based on the same assumptions made for the unstirred batch reactor; additional assumptions must, however, be introduced, specifically

1. Perfect mixing occurs in the reservoir.
2. Part of the enzyme, initially gelled on the ultrafiltration membrane, can be partially redissolved, because of the continuous recirculation of the solution.

The enzymatically catalyzed reaction takes place, therefore, also in the reservoir and in the fluid volume of the pipes and connectors.

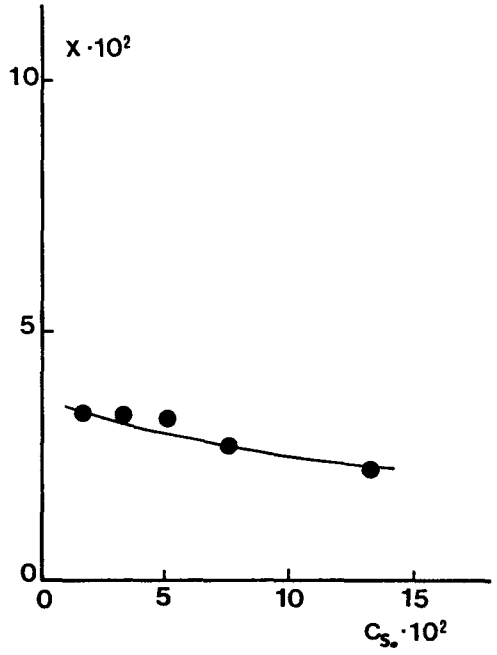

FIG. 6. Degree of conversion as a function of initial substrate concentration. Enzyme, urease (4×10^{-5} g/cm^2); $\Delta P = 2$ atm; $T = 30$°C; full line, analytical prediction.

In addition, it is assumed that the kinetics of the homogeneous reaction can be described by the classical Michaelis–Menten equation, using the parameters V_{max} and K_M determined in the homogeneous reaction system.

Equations 9 and 10 are derived from a mass balance in the reservoir for the substrate concentration, C_s, and for the product concentration, C_p, respectively:

$$(Q_T/V_s)C_s + R_s = -\mathrm{d}C_s/\mathrm{d}t \tag{9}$$

$$(Q_T/V_s)C_p + R_p = -\mathrm{d}C_p/\mathrm{d}t \tag{10}$$

In this case a simple stoichiometric relationship does not exist between C_s and C_p since chemical reaction in the reservoir is complicated by mass transport of substrate across the membrane.

Using these assumptions, the expression of the reaction rates, R_s, and R_p, is

$$R_s = -R_p = V_{max}C_s/(K_M + C_s) \tag{11}$$

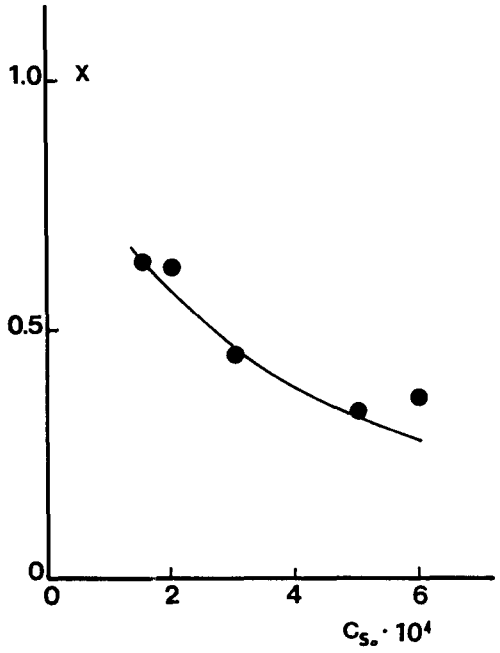

FIG. 7. Degree of conversion as a function of initial substrate concentration. Enzyme, glucosidase (1.2×10^{-6} g/cm^2); $P = 2$ atm; $T = 30$°C; full line, analytical prediction.

From equations 9, 10, and 11, and using the boundary conditions

$$\text{at } t = 0 \quad \begin{cases} C_p = 0 \\ C_s = C_{s0} \end{cases}$$

relationships for the concentration variations in the reservoir can be derived in dimensionless form:

$$-\ln(1 - X_1) + \alpha \ln\{1 - [\beta X_1/(1 + \alpha + \beta)]\} = (1 + \alpha)\tau \tag{12}$$

$$X_1^* = X_1 - [1 - \exp(-\tau)] \tag{13}$$

where

$$X_1 = (C_{s0} - C_s)/C_{s0}$$

$$X_1^* = (C_p/C_{s0})$$

$$\tau = (Q_T/V_s)t$$

$$\alpha = (V_{\max}/K_M)(V_s/Q_T)$$

$$\beta = C_{s0}/K_M.$$

The degrees of conversion in the reservoir, X_1 and X_1^*, can be calculated as a function of time, τ, enzyme amount, α, and initial substrate concentration, β, using equations 12 and 13 and the values of K_M and V_{max} determined from the homogeneous reactions. In the mass balance across the enzyme gel layer, the substrate concentration C_s is not assumed to be equal to the initial value, C_{s0}, since the reaction is proceeding in the reservoir as well as at the membrane surface. Assuming, however, that the time dependence of C_s is not significant, the system can be modeled as a pseudo-steady-state process. Following the same procedure used for deriving equation 6, an analogous expression can be derived:

$$-K'_M \ln\{1-[(X_2-X_1)/(1-X_1)]\}+C_{s0}(X_2-X_1)=\nu P_E/Q_T \qquad (14)$$

which correlates the degree of conversion measured in the permeate, X_2, and the degree of conversion measured in the reservoir, X_1, with the ultrafiltration flow rate, Q_T, the initial substrate concentration, C_{s0}, and the amount of enzyme, P_E. The degrees of conversion X_2 and X_2^* are given, respectively, by

$$X_2=(C_{s0}-C'_s)/C_{s0}$$

$$X_2=(C'_p/C_{s0})$$

The stoichiometric relationship between the substrate concentration and the product concentration upstream and downstream from the gel may be written in the dimensionless form

$$X_2-X_1=X_2^*-X_1^* \qquad (15)$$

Using equations 12 and 15 and the values of K_M and V_{max}, the experimental data can be compared with the analytical prediction.

To verify the analytical model, some experimental runs were carried out using the apparatus described in Fig. 7. The enzyme used was always acid phosphatase, which was chemically stable in the gelled form (10). After more than 150 hr, no significant activity decay was observed.

The effect of initial substrate concentration, applied pressure, axial flow rate, and time on the degree of conversion in the reservoir and in the permeate has been studied. In Fig. 8 a comparison between experimental and theoretical data describing the degree of conversion as function of time, at different initial substrate concentrations, is presented. The assumption of pseudo steady state used in deriving equation 14 is consistent with the data obtained here. The variation in the reaction rate within the gel due to the increase in substrate concentration upstream from the gel was, in this case, negligible. The dependence of the degrees of conversion X_1, measured in the reservoir, and X_2, measured in the permeate, is presented as function of

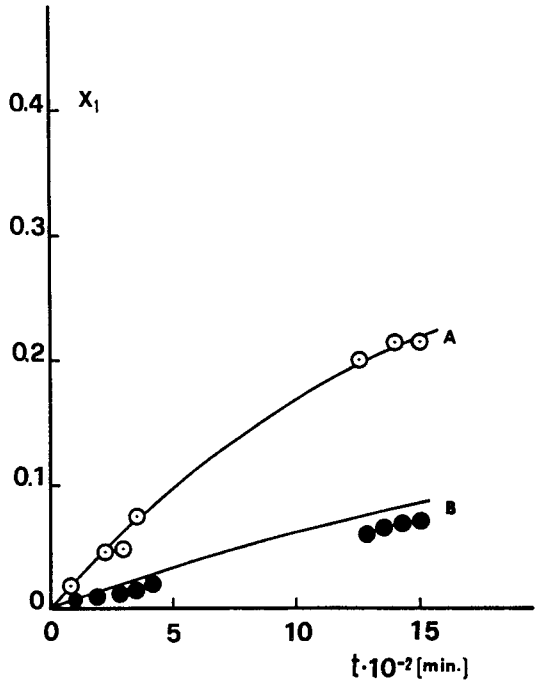

FIG. 8. Degree of conversion as a function of time in a recirculating UF reactor. Full line, analytical prediction; enzyme, acid phosphatase (4×10^{-6} g/cm^2); $\Delta P = 1.5$ atm; $T = 30$°C; A, $C_{s0} = 6 \times 10^{-4}$ M; B, $C_{s0} = 6 \times 10^{-5}$ M.

initial substrate concentration in Fig. 9. Also, in this case a significant agreement between the experimental data and analytical predictions has been observed. The data reported have been measured after 24 hr to ensure attainment of the steady state.

DISCUSSION AND CONCLUSION

In general, the agreement between the experimental results and the predictions of the simple analytical model of the two classes of enzymatic heterogeneous reactors is satisfactory.

As shown in Figs. 3, 5, and 6, the model adequately predicts the behavior of all three enzymes studied. Similar results have been obtained for the recirculating reactor (Fig. 8), relating the degree of conversion to initial substrate concentration (Figs. 3, 5, 6, and 9), pressure (Fig. 4), enzyme amount (Fig. 3), and time.

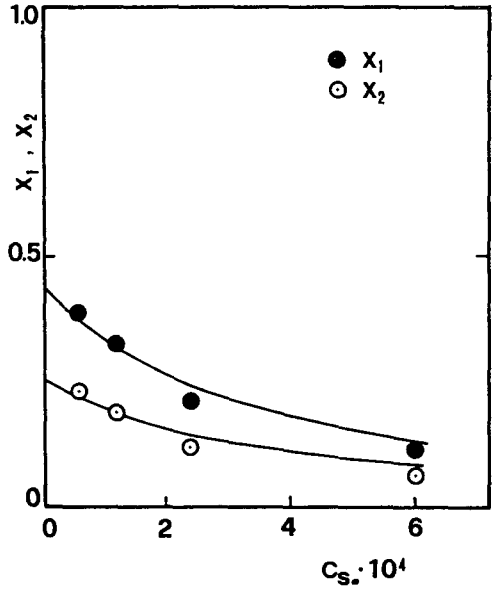

FIG. 9. Degrees of conversion X_1 (reservoir) and X_2 (permeate) as a function of initial substrate concentration at $t = 24$ hr. Full line, analytical prediction; enzyme, acid phosphatase; $\Delta P = 1.5$ atm; $J = 5 \times 10^{-4}$ cm/sec.

In the case of the continuous recirculating reactor, the kinetic parameters used in the derivation of the analytical curves were the same as those calculated previously in the batch heterogeneous gel membrane reaction (K'_M, V'_{max}). The values of K_M and V_{max} measured in the reservoir were equal to the ones measured independently in traditional homogeneous reactor.

The effect of axial flow rate on the degree of conversion (as shown in Fig. 10) is qualitatively consistent with the theoretical analysis. In fact, the increase of the degree of conversion in the reservoir may be attributed to a decrease in the amount of the enzyme which is gelled on the membrane, therefore increasing the enzyme concentration in the bulk.

The value of the "apparent" Michaelis constant K'_M, obtained through the integral method of analysis used in these analytical models, is different from the values which can be obtained by a standard double reciprocal plot presented, for example, by Lineweaver and Burk (11). This discrepancy, however, clearly is due to the deviations from the Lineweaver–Burk model for heterogeneous gelled enzyme reactors where the concentrations of the substrate upstream and downstream from the gelled enzyme layer are significantly different. The Lineweaver–Burk assumption of constant reac-

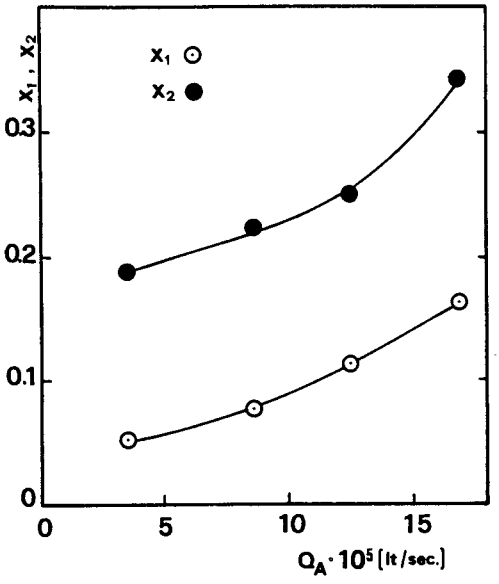

FIG. 10. Degrees of conversion X_1, X_2 as a function of axial flow rate. Enzyme, acid phosphatase; $C_{s0} = 6 \times 10^{-5}$ M; $\Delta P = 1.5$ atm.

tion rate throughout the gel is an oversimplification which is not valid here because the reaction rate is generally concentration dependent. In practice, this type of heterogeneous reactor cannot be treated by a differential method of analysis required by the Lineweaver–Burk method.

A tentative analysis of the productivity of this type of reactor is rather difficult. In fact, the traditional productivity, defined as moles converted per unit time per unit of catalyst, is not the most appropriate parameter. Factors including enzyme stability, enzyme recovery and reuse, purity of the effluent products, and problems related to the enzyme inhibition by products should be considered. If these factors are not considered, the productivity of enzyme gel reactors appears to be lower than the productivity measured in homogeneous systems. This result may be attributed to steric factors associated with accessibility of the active sites in the dense gel or/and to electrostatic interactions between substrate and gel. If enzyme stability is introduced into the definition of productivity, the heterogeneous reactors described here are more satisfactory.

All of the other parameters not considered in the productivity definition positively affect the heterogeneous ultrafiltration reactor. The effect of the external diffusion on the total rate of reaction may be considered, in these experimental runs, to be negligible (in concert with the hypothesis of

uniform concentration of substrate upstream of the gel layer). In fact, the values of the kinetic constants for the immobilized enzyme were the same in the recirculating and in the unstirred batch reactor, and these values were not dependent on the axial flow rate.

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