

THEORETICAL AND EXPERIMENTAL ANALYSIS OF AN IMMOBILIZED ENZYME MEMBRANE REACTOR

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The unsteady state behavior of an immobilized enzyme membrane reactor has been analyzed both theoretically and experimentally. The reacting system considered is sucrose hydrolysis by invertase (EC 3.2.1.26). Enzyme immobilization has been obtained by ultrafiltering co-cross-linked invertase through a cellulose acetate asymmetric membrane. On the basis of the proposed mathematical model, the steady state behavior of the system can be dealt with in terms of a continuous stirred tank reactor

INTRODUCTION

Recently, the possibility of combining an enzyme and an asymmetric artificial membrane as a catalytic unit operating in an ultrafiltration (UF) system has been reported (1,2). As an alternative to this unit we have considered the use of a copolymerized enzyme composite membrane obtained by co-cross-linking invertase (EC 3.2.1.26) to an inert protein and immobilizing the resulting soluble bound enzyme onto the active surface of a UF membrane. In this paper we present a mathematical model for the enzyme reactor (IEMR) formed by such a composite membrane operating in an unstirred UF cell continuously fed with sucrose, i.e., the invertase substrate. Invertase has been chosen both for the ease with which its catalytic activity can be measured and for its potential applications. The

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basic goal of the mathematical model which has been set up is to allow the interpretation of the steady state kinetic results in order to compare the behavior of the immobilized enzyme to that of the soluble one. The validity of the proposed model has been confirmed by its capability to predict correctly the unsteady state behavior of the system.

EXPERIMENTAL

Description of the Apparatus

This consists of an unstirred UF cell (50 ml, 4 cm inner diameter) with an outer jacket in which temperature-controlled water can be circulated to maintain a temperature of $30 \pm 0.1^\circ\text{C}$, a fiberglass or stainless steel reservoir for the substrate solution, and an automatic fraction collector.

Kinetic Assay

The reaction is started by applying a nitrogen gas pressure of 2 atm to the reservoir containing sucrose in 0.1 M acetate buffer, pH 4.65, after connecting the latter to the UF cell. Fractions of permeate were collected at 3-min intervals, samples thereof were taken and analyzed for reducing power by the colorimetric method of Nelson (3), and the results were expressed in terms of glucose (mol/ml). The UF rate was measured by means of a capillary flow meter. At the end of each run the enzyme composite membrane was thoroughly washed with distilled water until no reducing power could be detected in the permeate, then stored in the assembled UF cell at 4°C to avoid microbial contamination.

Immobilization of Invertase

Invertase (Boehringer, from yeast) was first co-cross-linked to bovine serum albumin (Sigma, crystal) by a procedure essentially similar to that described by Paillot et al. (4). Ten milliliters of a solution containing 315 mg of bovine serum albumin, 10 mg invertase, and 42.5 mg glutaraldehyde (Serva, 25% solution) in 0.02 M phosphate buffer, pH 6.8, was prepared and kept at 4°C for 18 h. The soluble copolymerized invertase thus obtained was stabilized by addition of glycine up to a final concentration of 2% and dialyzed for 24 h at 4°C against a 1% glycine solution in the phosphate buffer. Then, 0.5 ml of the stabilized soluble bound invertase was diluted with 9.5 ml of the phosphate buffer and ultrafiltered overnight at 1 atm until a very thin layer of copolymerized invertase was formed, which was tightly adherent to the UF membrane (DDS 600, The

Danish Sugar Corporation). This operation was performed in the same UF cell used throughout all the experiments.

THEORETICAL SECTION

In the dynamic analysis, the UF cell has been divided into two portions: upstream and downstream the membrane-supported enzyme layer, respectively. In order to obtain the overall theoretical system transient response, attention has to be paid to both parts and suitable models are to be developed.

Upstream Portion of the UF Test Cell

As far as this region is concerned, it has to be regarded as a distributed parameters system and hence its mathematical model is to be essentially based on the usual mass transfer balance equations. Owing to the low flow rates attained in the experimental runs, both convection and diffusion have to be taken into account.

Since the diffusion coefficients for sucrose, fructose, and glucose are close to each other, within less than 30%, a single diffusion coefficient has been assumed for substrate and products. Furthermore, the membrane molecular weight cutoff (20,000) ensures no rejection of sucrose, fructose, and glucose, and hence that no polarization phenomena take place. This, together with the low conversion levels attained, warrants that the variation in substrate and products concentrations is limited enough to enable one to assume the diffusion coefficient to be constant. Hence, the mass balance equations can be written in dimensionless form:

$$\frac{\partial^2 \gamma_s}{\partial \xi^2} + \frac{\partial \gamma_s}{\partial \xi} = \frac{\partial \gamma_s}{\partial \tau} \quad (1)$$

$$\frac{\partial^2 \gamma_p}{\partial \xi^2} + \frac{\partial \gamma_p}{\partial \xi} = \frac{\partial \gamma_p}{\partial \tau} \quad (2)$$

with the obvious initial condition:

$$\text{I.C.} \quad \tau = 0, \quad \forall \xi, \quad \gamma_s = 1, \quad \gamma_p = 0$$

$\tau = 0$ has been assumed as the time at which pressure is applied to the feed vessel.

In order to identify suitable boundary conditions, the order of magnitude of the dimensionless depth of penetration ($\xi = 1$), i.e., the height of the upstream volume in which the substrate and products concentrations

appreciably differ from the corresponding feed values, has to be determined. For the experimental conditions adopted, this is roughly equal to 4×10^{-2} cm. If a comparison is made with the total cell height (5 cm), the upstream region can be obviously regarded as infinite along the ξ axis. Hence, the first boundary condition can be written as

$$\text{B.C. 1} \quad \xi \rightarrow \infty, \quad \forall \tau, \quad \gamma_s = 1, \quad \gamma_p = 0$$

On the basis of the amount of enzyme deposited onto the membrane surface, the thickness of the enzymatic layer can be roughly estimated as 5×10^{-5} cm, which is negligible as compared to the previously evaluated characteristic dimension of the system.

Therefore, the enzymatic reaction can be considered to take place on the free surface of the enzyme layer only. This, together with the membrane rejection coefficient toward both substrate and products being zero, leads to the second boundary condition:

$$\text{B.C. 2} \quad \xi = 0, \quad \forall \tau, \quad \frac{\partial \gamma_s}{\partial \xi} - \rho = \frac{\partial \gamma_p}{\partial \xi} + \rho = 0$$

As far as the reaction term per unit membrane surface area ρ is concerned, only the two limiting cases for first-order and zero-order kinetics have been considered:

$$\rho = \alpha_1 \gamma_s \quad (\text{first-order kinetics})$$

$$\rho = \alpha_0 \quad (\text{zero-order kinetics})$$

Indeed, it did not seem worthwhile to introduce an only apparently more rigorous kinetic equation such as a Michaelis–Menten one. This is mainly because, although for sucrose inversion in a soluble enzyme reactor a modified Michaelis–Menten equation has been shown to hold, it can hardly be expected that the same reaction rate expression and the same values of the kinetic parameters will apply in an immobilized enzyme layout as the one considered. On the other hand, a Michaelis–Menten equation, because of its intrinsic nonlinearity, would definitely rule out the possibility of obtaining a closed-form expression for the model theoretical solution.

Downstream Portion of the UF Test Cell

Specific consideration has been devoted to the analysis of the dynamics of the downstream part of the UF cell. In fact, although the enzymatic reaction can only take place on the membrane surface and no further chemical transformation occurs in the permeate stream, the volume

of the downstream region is of the same order of magnitude as the upstream volume involved in substrate and products penetration (~ 1 ml).

The identification was carried out by first step testing the enzyme reactor in the absence of reaction according to the following procedure. The UF cell was washed through with the acetate buffer solution for about 1 h; pressure was applied directly to the UF cell until complete emptying of the upstream portion was achieved. Glucose, one of the reaction products, at 1 mM concentration in the same buffer solution was then fed to the cell and pressure restored. The permeate was collected in the same way as for the reaction products. Glucose concentration versus collection time is reported in Fig. 1 in terms of data points.

The response diagram obtained clearly indicates that partially segregated plug flow occurs and that the downstream system, therefore, can be modeled in terms of a first-order lag (stirred tank) in series with a pure time delay.

The corresponding transfer function which relates the dimensionless product concentration at the UF test cell outlet to that immediately downstream the membrane is then

$$\frac{\tilde{\gamma}_{pE}(s)}{\tilde{\gamma}_p(0, s)} = \frac{e^{-\tau_1 s}}{1 + \tau_2 s}$$

The best fit of the experimental data was found for values of T_1 and T_2 (time delay and system time constant, respectively) which, together with the flow rate adopted in the experimental run, lead to the following evaluations:

$$V_1 = 0.18 \text{ ml} = \text{time delay volume}$$

$$V_2 = 0.69 \text{ ml} = \text{segregated region volume}$$

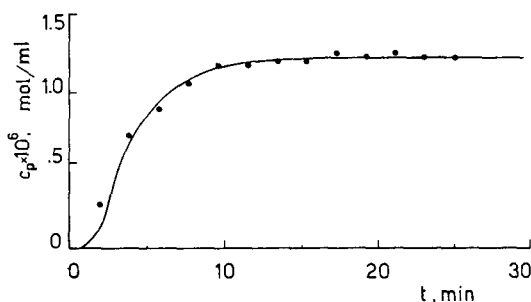


FIG. 1. Step response of the downstream portion of the test UF cell. For experimental details, see text.

These values were assumed to be characteristic of the system and were therefore employed in all the experimental runs whatever the flow rate.

It has to be noticed that, in order to make the theoretical results comparable with the experimental ones, the model parameter estimation procedure has been carried out taking into account the finite collection time interval (see Fig. 1, solid line).

Overall System Response

The solutions of Eqs. (1) and (2) evaluated at $\xi = 0$ in the Laplace domain times the downstream transfer function just defined directly yield the overall Laplace domain system response $\bar{\gamma}_p^*(s)$:

$$\bar{\gamma}_p^*(s) = \frac{\alpha_1 e^{-\tau_1 s}}{s(1 + \tau_2 s)(\alpha_1 + 0.5 + \sqrt{s + 0.25})} \quad (\text{first-order kinetics}) \quad (3)$$

$$\bar{\gamma}_p^*(s) = \frac{\alpha_0 e^{-\tau_1 s}}{s(1 + \tau_2 s)(0.5 + \sqrt{s + 0.25})} \quad (\text{zero-order kinetics}) \quad (4)$$

Inversion of Eq. (3) gives

$$\begin{aligned} \gamma_p^*(\tau) = & \left\{ \frac{\alpha_1 + 0.5}{\alpha_1 + 1} (1 - e^{-\tau^*/\tau_2}) - \frac{0.5}{\alpha_1 + 1} \operatorname{erf} \sqrt{0.25 \tau^*} \right. \\ & + \frac{(\alpha_1 + 0.5) e^{-\tau^*/\tau_2}}{\tau_2(\alpha_1 + 1)(\alpha_1^2 + \alpha_1 + \tau_2^{-1})} [1 - \exp((\alpha_1^2 + \alpha_1 + \tau_2^{-1})\tau^*) \\ & \times \operatorname{erfc}((\alpha_1 + 0.5)\sqrt{\tau^*})] + \Phi(\tau^*) \Big\} u(\tau^*) \quad (\text{first-order kinetics}) \quad (5) \end{aligned}$$

where

$$\Phi(\tau^*) = \frac{\alpha_1 e^{-\tau^*/\tau_2} \operatorname{erf}(\sqrt{(0.25 - \tau_2^{-1})\tau^*})}{(\alpha_1^2 + \alpha_1 + \tau_2^{-1})} \sqrt{(0.25 - \tau_2^{-1})}$$

if $\tau_2 > 4$, and

$$\Phi(\tau^*) = -\frac{2\alpha_1 \sqrt{\tau_2^{-1} - 0.25} e^{-0.25\tau^*}}{\sqrt{\pi}(\alpha_1^2 + \alpha_1 + \tau_2^{-1})} \psi(\sqrt{(\tau_2^{-1} - 0.25)\tau^*})$$

if $\tau_2 < 4$.

Inversion of Eq. (4) provides

$$\begin{aligned} \gamma_p^*(\tau) = & \alpha_0 [0.5(\tau_2 - \tau^*) \operatorname{erfc} \sqrt{0.25 \tau^*} + \operatorname{erf} \sqrt{0.25 \tau^*} \\ & + \sqrt{\frac{\tau^*}{\pi}} e^{-0.25\tau^*} - 0.5\tau_2 e^{-\tau^*/\tau_2} + \Psi(\tau^*)] u(\tau^*) \quad (\text{zero-order kinetics}) \quad (6) \end{aligned}$$

where

$$\Psi(\tau^*) = \frac{0.25\tau_2}{\sqrt{0.25 - \tau_2^{-1}}} e^{-\tau^*/\tau_2} \operatorname{erf} \sqrt{(0.25 - \tau_2^{-1})\tau^*}$$

if $\tau_2 > 4$, and

$$\Psi(\tau^*) = \frac{0.5 e^{-0.25\tau^*}}{\sqrt{\tau_2^{-1} - 0.25}} \cdot \frac{\tau_2}{\sqrt{\pi}} \psi[\sqrt{(\tau_2^{-1} - 0.25)\tau^*}]$$

if $\tau_2 < 4$.

RESULTS AND DISCUSSION

In Table 1 are listed the characteristic parameters of a series of experimental runs carried out under the same physical conditions at different sucrose concentrations in the feed stream.

The experimental results in terms of glucose are reported as a function of reaction time (Figs. 2-5), $t = 0$ corresponding to the time at which pressure is applied to the feed reservoir.

Knowledge of the experimental conditions (see Table 1) allows the evaluation of all the parameters involved in the determination of the theoretical system response [Eqs. (5) and (6)] to be performed, except α_1 and α_0 .

On the other hand, the basic purpose of the current paper is to set up a reliable mathematical model of a simple kinetic system, such as the one considered, in order to make comparisons between the kinetics of the immobilized system and the corresponding soluble enzyme system.

The ultimate value of the dimensionless product concentration can be related to the kinetic parameters α_1 and α_0 via either Eqs. (3) and (4),

TABLE 1. Sucrose Hydrolysis by UF Membrane-Supported Immobilized Invertase^a

Run	$Q \times 10^3$	$C_s^0 \times 10^6$	$C_{p\infty} \times 10^6$	α_0	α_1	τ_1	τ_2	$k_0 \times 10^{10}$	$k_1 \times 10^{10}$
144	1.36	2	1.77	0.888	7.89	0.412	1.58	1.92	8.52
184	1.26	3	2.05	0.683	2.45	0.380	1.47	2.05	2.45
214	1.11	4	3.17	0.792	4.00	0.338	1.29	2.80	3.54
274	1.13	1.5	1.35	0.900	14.00	0.351	1.31	1.22	12.61

^a Experimental conditions: enzyme, 0.398×10^{-5} g/cm²; membrane, DDS600; membrane cross-sectional area, 12.57 cm²; temperature, $30 \pm 0.1^\circ\text{C}$; pressure, 2 atm; pH 4.65; diffusivity, 3.74×10^{-6} cm²/sec.

respectively, or an overall steady-state mass balance yielding

$$\lim_{\tau \rightarrow \infty} \gamma_p^*(\tau) = \frac{\alpha_1}{\alpha_1 + 1}, \quad \lim_{\tau \rightarrow \infty} \gamma_p^*(\tau) = \alpha_0$$

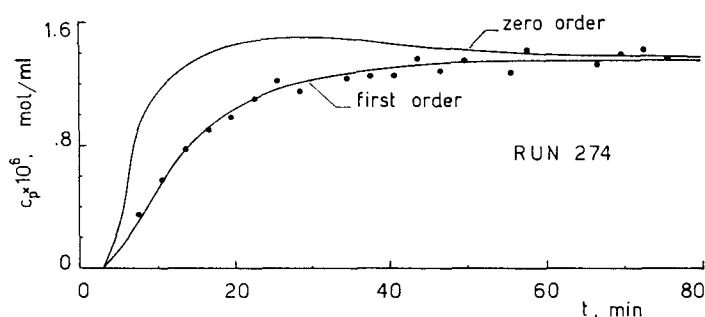


FIG. 2. Hydrolysis of sucrose (1.5×10^{-6} mol/ml) by immobilized invertase operating in an IEMR. Comparison between experimental data (expressed in terms of glucose) and theoretical curves (solid lines) calculated according to Eqs. (5) and (6). For experimental details, see Table 1.

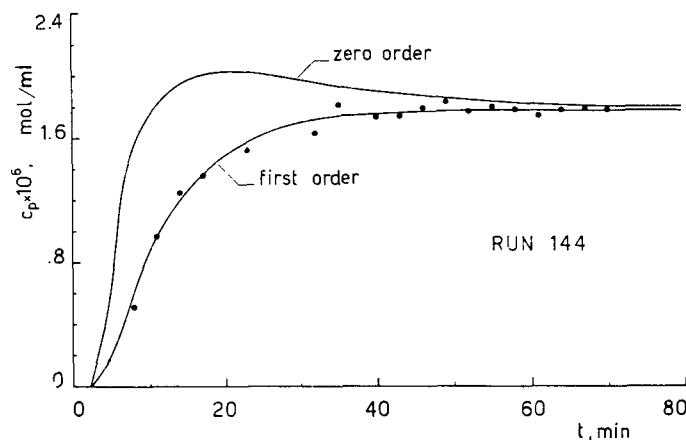


FIG. 3. Hydrolysis of sucrose (2×10^{-6} mol/ml) by immobilized invertase operating in an IEMR. Comparison between experimental data (expressed in terms of glucose) and theoretical curves (solid lines) calculated according to Eqs. (5) and (6). For experimental details, see Table 1.

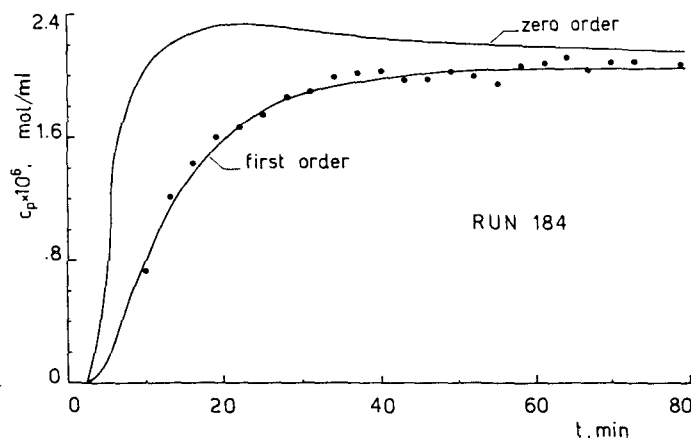


FIG. 4. Hydrolysis of sucrose (3×10^{-6} mol/ml) by immobilized invertase operating in an IEMR. Comparison between experimental data (expressed in terms of glucose) and theoretical curves (solid lines) calculated according to Eqs. (5) and (6). For experimental details, see Table 1.

Therefore, α_1 and α_2 have been obtained on the basis of the experimental values of steady state product concentrations.

Once the theoretical response has been obtained the results are worked out using the same procedure as the one adopted in dealing with the downstream system dynamics, to take into account the discrete sampling time intervals. The corresponding curves are reported in Figs. 2–5 (solid lines).

These figures clearly show a remarkable agreement between theoretical predictions for first-order kinetics and experimental results for each individual run, and hence the proposed mathematical model adequately describes the system. However, it has to be noticed that the first-order kinetic constants determined for each run depend on substrate concentration in the feed. This, in turn, implies that a more complex rate expression holds for the whole range of substrate concentrations investigated and that the pseudo-first-order kinetic constant experimentally determined through the steady state value of the products concentration has to be regarded as the result of a local linearization of the true system kinetics. Further experimental analyses on the immobilized enzyme kinetics are underway.

CONCLUSION

The mathematical model described in this paper has been shown to predict correctly the unsteady state behavior of the immobilized enzyme

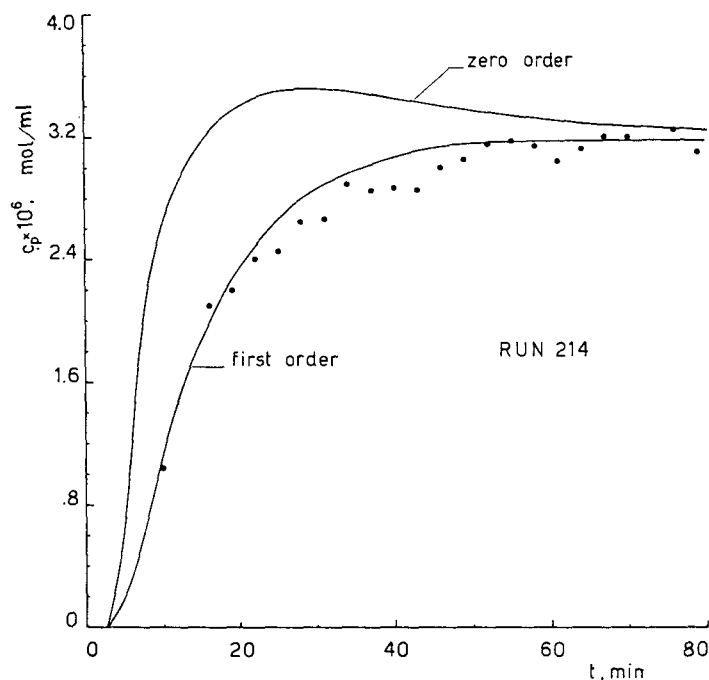


FIG. 5. Hydrolysis of sucrose (4×10^{-6} mol/ml) by immobilized invertase operating in an IEMR. Comparison between experimental data (expressed in terms of glucose) and theoretical curves (solid lines) calculated according to Eqs. (5) and (6). For experimental details, see Table 1.

membrane reactor (IEMR) on which the experimental runs have been carried out. This allows the steady state reaction rate values to be related to well-defined substrate and product concentrations. It has to be pointed out that, because the thickness of the enzyme layer is negligible as compared to reactant and products depth of penetration and because the membrane rejection coefficient toward both reactant and products is zero, reactant and product concentrations at the enzyme surface coincide with the outlet values. Therefore, the steady state system can be dealt with as a whole in terms of simple continuous stirred tank reactor.

The IEM reactor described in this paper, apart from the well-known advantages compared to a soluble enzyme reactor, as far as enzyme stability and recovery are concerned, could be even more usefully adopted with a suitable choice of the membrane molecular weight cutoff. Indeed, if the membrane exhibited reasonably high rejection toward the substrate and complete product permeability, this system could, on one hand, handle

considerably diluted substrate solutions thanks to polarization effects, and on the other hand contribute to product separation from substrate.

Furthermore, the IEMR configuration seems to be more suitable to applications to flow systems than the other enzyme membrane reactors proposed in the literature, in which only a gelified enzyme layer is obtained over the membrane active surface, obviously prone to mechanical removal by the flowing stream.

NOTATION

C	Concentration, mol/ml
D	Diffusion coefficient, cm^2/sec
k_0	Zero-order rate constant per unit surface, $\text{mol}/\text{cm}^2 \text{ sec}$
k_1	First-order rate constant per unit surface, cm/sec
Q	Flow rate, ml/sec
s	Laplace parameter, dimensionless
t	Time, sec
T_1	Downstream time constant (time delay), sec
T_2	Downstream time constant (segregated region), sec
$u(t)$	Unit step function, dimensionless
v	Fluid velocity, cm/sec
V_1	Downstream delay volume, ml
V_2	Downstream segregated volume, ml
x	Space coordinate, cm

Dimensionless variables

$\alpha_0 = k_0/C_s^0 v$	Zero-order kinetic parameter
$\alpha_1 = k_1/v$	First-order kinetic parameter
$\gamma = C/C_s^0$	Concentration
$\tilde{\gamma}^*(s)$	Laplace transform of the overall system response
$\xi = x \cdot v/D$	Space coordinate
$\psi_{(z)} = e^{-z^2} \int_0^z e^{u^2} du$	Dawson's integral
$\tau = t \cdot v^2/D$	Time
$\tau^* = \tau - \tau_1$	Time
$\tau_1 = T_1 v^2/D$	Downstream time constant (time lag)
$\tau_2 = T_2 v^2/D$	Downstream time constant (segregated region)

Subscripts

- E Cell outlet
- p Product
- s Substrate
- ∞ Steady state value

Superscripts

- ⁰ Initial value
 - Laplace transformed variable
 - *
- Overall system response

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REFERENCES

1. DRIOLI, E., GIANFREDA, L., PALESCANDOLO, R., and SCARDI, V. (1975) *Biotechnol. Bioeng.* 17 : 1365.
2. DRIOLI, E., GIANFREDA, L., PALESCANDOLO, R., and SCARDI, V. (1976) *In* Analysis and Control of Immobilized Enzyme Systems, THOMAS, D., and KERNEVEZ, J. P. (eds.), North-Holland Publ., Amsterdam, p. 179.
3. NELSON, N. (1944) *J. Biol. Chem.* 153 : 375.
4. PAILLOT, B., REMY, M. H., THOMAS, D., and BROUN, G. (1974) *Pathol. Biol.* 22 : 491.
5. HODGMAN, C. D. (ed.) (1963) *Handbook of Chemistry and Physics*, CRC Press, Cleveland, Ohio.