報 文
화학공학 제11권, 제3호
J. KIChE. Vol. 11, No. 3
June 1973

Simulation and Optimization of Penicillin Amidohydrolase Enzyme Reactor System

**D. Y. Ryu · **B. K. Lee

* *Department of Chemical Engineering Massachusetts Institute of Technology Cambridge, Massachusetts, U. S. A.*

** *Schering Corporation Bloomfield, N. J., U. S. A.*

(접수 11. 30. 1972)

Summary

The activity of penicillin amidase obtained from *B. megaterium* and *M. roseus* were compared with that obtained from other microbial sources. By using both the soluble and immobilized forms of the enzyme obtained from *B. megaterium*, we have studied the reaction kinetics. The products of the enzymatic hydrolysis of benzylpenicillin, PAA and 6-APA, have been found to be competitive and noncompetitive inhibitors of the enzyme, respectively. We have developed a kinetic model of the reaction based on this finding and a mathematical model for a continuous enzyme reactor system was formulated. With the aid of a computer, simulation studies of the enzyme reactor systems were carried out, and the results compared favorably with the experimental results. The productivity (defined as moles of product formed per liter of reactor volume per hour) was optimized with respect to the space time of the reactor system and the concentration of the substrate in the feed. It was found that the productivity of the immobilized enzyme reactor system was more favorable as compared with the soluble enzyme reactor system under certain operating conditions.

Introduction

Microbial sources of penicillin amidase (benzylpenicillin amidohydrolase, EC 3. 5. 1. 11) are widely used in the production of semisynthetic penicillins. This enzyme hydrolyzes benzylpenicillin to yield 6-aminopenicillanic acid (6-APA) and phenylacetic acid (PAA). This reaction of enzymatic hydrolysis is shown in Fig. 1. This enzyme is of considerable commercial importance, since many semisynthetic penicillins are prepared from 6-APA.

Some semisynthetic penicillins, as 6-APA derivatives, have better characteristics than benzylpenicillin in terms of efficacy and clinical value. Some of these desirable characteristics are shown in Table 1. The structure-activity relationship of 6-APA derivatives has been well summarized by Price.[1]

Several species of microorganisms, as well as some plant and animal tissues, are very good sources of penicillin amidase.[2-15]

Table 1. Desirable Characteristics of Semisynthetic Penicillins

1. A greater degree of intrinsic antibiotic activity than the benzylpenicillin.
2. A wider spectrum than that possessed by benzylpenicillin.
3. Stability in an acidic medium.
4. Intestinal absorbability and orally administrable.
5. Low degree of deleterious binding to serum protein.
6. Reduced allergenicity from benzylpenicillin.
7. Resistance to the action of microbial β-lactamase.

Among these, the enzymes isolated from or produced by *Bacillus megaterium* (ATCC 14945) and *Micrococcus roseus* (ATCC 516) have been found to give good hydrolytic activity. The enzymes obtained from these two bacterial sources have been compared.

We have studied the reaction kinetics of the enzy-

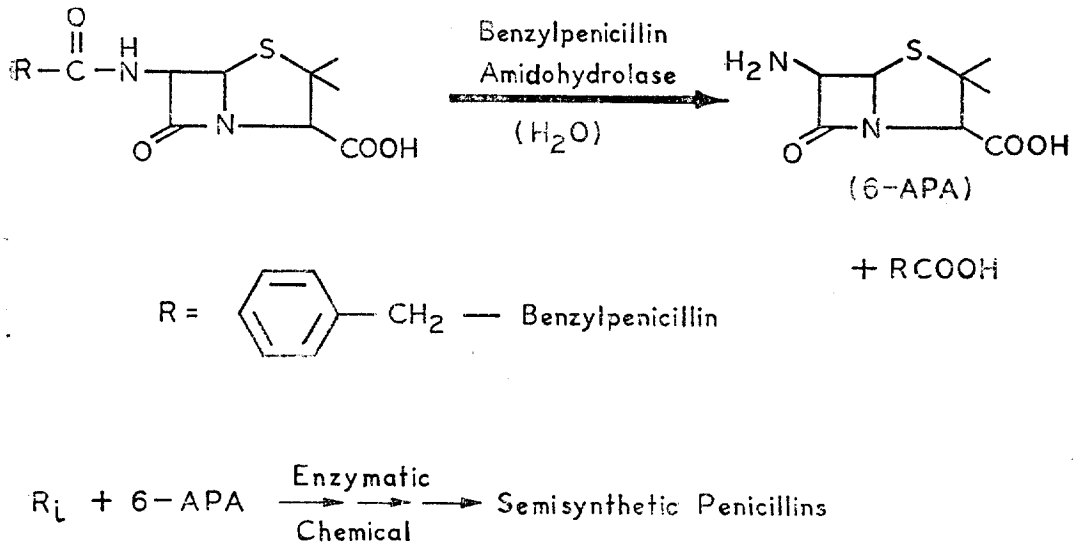

Fig. 1 Hydrolysis of penicillin by benzylpenicillin amidohydrolase, Enzymatic preparation of 6-aminopenicillanic acid (6APA)

matic hydrolysis of benzylpenicillin to determine the kinetic constants. In this enzymatic reaction, both products of hydrolysis, 6-APA and PAA, are inhibitors of the reaction, 6-APA noncompetitively and PAA competitively. A kinetic model was derived that was based on the double inhibition effect of the products on the enzymatic reaction. This kinetic model was then used, with the aid of a computer, to simulate the performance of a continuous enzyme reactor system and to optimize the productivity of the reactor system in terms of process variables.

Experimental

1) Enzyme preparation from *B. megaterium*. The culture of *B. megaterium* (ATCC 14945) was grown in 500-ml flasks according to the method described by Murao, Sakaguchi, and Kono[10] and by Heuser, Chiang, and Anderson.[16] The medium consisted of enzyme-hydrolyzed casein (produced by Amber Lab., Inc., Milwaukee, Wisconsin), 3%; glucose, 0.5%; and Ucon antifoam LB625, 0.01% (produced by Dow Chemical Co., Midland, Michigan). The pH was adjusted to 7.0 before and after sterilization. About 0.15% (by weight) of phenylacetic acid was added as an inducer of the enzyme. After about 48 to 72 hours of incubation, the whole broth was treated with 0.5% (v/v) Primafloc C-3 (a flocculating agent, Rohm and Hass Co., Philadelphia, Penn.), 0.2% (v/v) toluene was added and mixed at room temperature, and the pH was readjusted to 7.0 to 7.5. The penicillin amidase from *B. megaterium* is an extracellular enzyme, and the centrifuged supernatant can be used as a crude form of the soluble enzyme.

For preparation of the immobilized enzyme, the centrifuged supernatant was treated with 0.3% (by weight) $CaCl_2$. After solution of the $CaCl_2$, a mixture of 1% (by weight) each of bentonite (U. S. P.) and Hyflo-Supercel (produced by Johns Manville, Manville, New Jersey) was added. The pH was adjusted to 6.2~6.3 with concentrated HNO_3, and agitation was continued for one hour at room temperature while the pH was maintained at 6.2~6.3. The mixture was then filtered on a Hyflo-precoated filter, and the penicillin amidase immobilized on bentonite was washed with 0.5% aqueous $CaCl_2$ solution.

The wet enzyme cake (penicillin amidase adsorbed on Hyflo-bentonite) was slurried in a mixture of water (40% of the original broth supernatant) and

0.1% toluene, the pH was adjusted to 8.0~8.2 with 10% NH_4OH, the mixture was warmed to 28°C, and the benzylpenicillin was added for hydrolysis.

2) Enzyme from *M. roseus*. The enzyme was prepared in flasks according to the method described by Pruess and Johnson.[14]

M. roseus (ATCC 516) was grown in 500-ml flasks containing 100 ml of medium, consisting of Beef Heart Infusion broth (Difco), 2.5%; glucose, 4%; K_2HPO_4, 0.95%; and KH_2PO_4, 0.6%. PAA was used as an inducer at a 0.15% concentration. Cells were centrifuged and washed with water, then washed twice with acetone (40% of initial broth volume). The acetone-washed cells were dried at moderate temperature and used as an acetone-dried whole-cell enzyme preparation.

3) Determination of enzymatic activity. The enzymatic activity of the penicillin amidase preparations was determined by a modified hydroxylamine method that is based on measurement of the products of the enzymatic hydrolysis, 6-APA and PAA, according to the methods described by Batchelor *et al.*[17] and by Niedermayer.[18]

The specific activity is defined as units of enzyme activity per mg of dry cell weight (for intracellular enzymes) and as units of enzyme activity per mg of protein (for extracellular enzymes). One unit of enzyme is defined as the activity of enzyme that is equivalent to one μ mol of product formed per minute under the specified conditions.

4) Purification of enzyme. To determine the kinetic constants accurately, the reaction rates were measured with both the crude and partially purified forms of enzyme obtained from *B. megaterium*. Purification was carried out by the method of Chiang and Bennett.[19]

The centrifuged supernatant liquid containing the enzyme was acidified with dilute acetic acid to pH 6.4, and was mixed with acid-washed Celite (Johns-Manville) at a ratio of 15 g per liter.

After being stirred for about two hours, the mixture was filtered and washed with water. The Celite cake was slurried in 24% (w/v) ammonium sulfate in 0.1 M tris-(hydroxymethyl)-aminomethane buffer (adjusted to pH 8.4 with NH_4OH) and was transferred to a Celite column.

Elution of the enzyme from the Celite column was carried out by the addition of ammonium sulfate solution. The eluate was concentrated by vacuum evaporation under mild conditions. The precipitated protein was separated by filtration and resuspended in 0.05 M phosphate buffer (pH 7.0). This suspension was dialyzed against a 50-fold volume of 0.025 M phosphate buffer (pH 6.4).

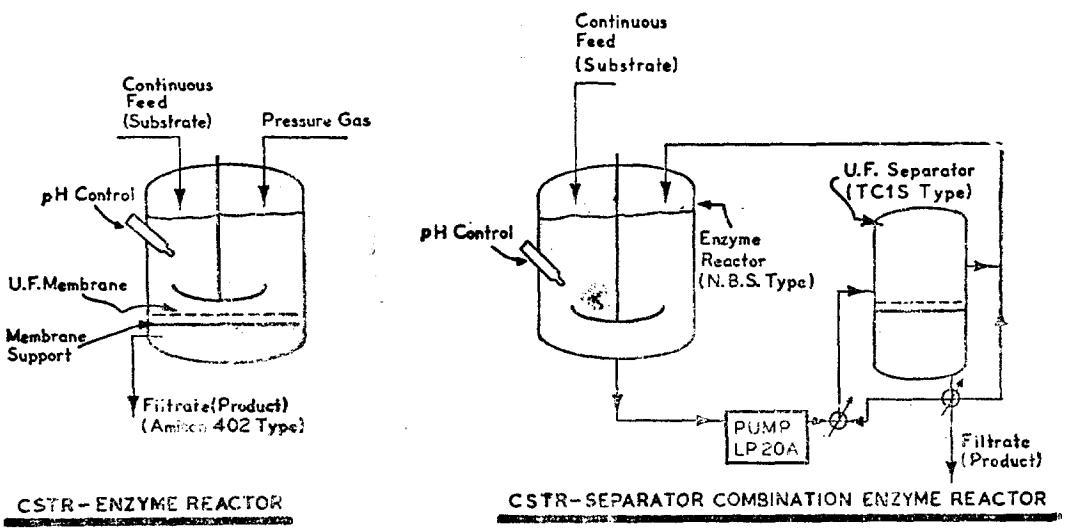

Fig. 2 The enzyme reactor systems used, Single-stage continuous stirred tank reactor (CSTR) with the ultra-filter membrane (Amicon 402 type reactor) and the enzyme reactor arrangement with enzyme recycle combined with ultra-filter separator (combination of New Brunswick Scientific's 2—L fermentor and Amicon TCIS type separator)

Further purification was carried out on a carboxymethylcellulose column in a cold room, followed by a second elution on a Celite column.

5) Operation of enzyme reactor systems. The hydrolysis of benzylpenicillin can be carried out at constant temperature (40°C) and pH (8.0), with a pH control. The enzyme can be reused in batch or continuous mode. The experimental arrangement is depicted in Fig. 2. A combination of enzyme reactor and ultrafilter (of the type manufactured by Amicon, Lexington, Mass., see Fig. 2 for models) was used as a continuous enzyme reactor system. Benzylpenicillin was fed continuously into the enzyme reactor, which had been charged with the penicillin amidase. When this system was operated, as a single-stage continuous stirred tank reactor (CSTR) system, the product was filtered out as a filtrate and the enzyme was retained within the reactor as a retentate. Both the soluble and immobilized forms of the enzyme could be used, since the bentonite-immobilized enzyme, in the form of fine particles, can be suspended readily by adequate agitation. When the system consisted of a combination of the enzyme reactor and thin-channel ultrafiltration unit, or its equivalent, the enzyme was recirculated and the product was filtered continuously.

The mean residence time could be adjusted by altering the flow rate of the substrate feed solution and the size of the reactor. Typical experimental conditions are given in Table 2.

Table 2. Typical Experimental Conditions

Mode of Operation: Continuous
Volume of Enzyme Reactor: 1 liter
Temperature: 40 ± 1°C
pH maintained and controlled: 8.0 ± 0.1
Agitation Speed: 400 RPM (single impeller)
Filtrate Flow Rate:
  170 ml/hr at time 0
  130 ml/hr at steady state
Ultrafiltration Membrane: Amicon UM 2, UM 10, and XM 50
Ultrafiltration Unit: Amicon thin-channel type
Recirculation Rate: 1.7 liter/min
Enzyme Loading: 3.5×10^4 units/liter
Specific Activity of Enzyme: 315 units/mg
Duration of run: 76 hr
Steady-state Conversion: 64~83%
Feed Concentration of Substrate: 2~5 gm/liter

Results and Discussion

1) Enzyme activity. The activity of penicillin amidase obtained from *B. megaterium* was compared with that of penicillin amidase obtained from other microbial sources. The enzyme activities found, as well as some reported in the literature, are shown in Table 3. All the organisms listed in Table 3 appear to be good sources of penicillin amidase. The selection of a particular microbial source of the enzyme will depend, in large part, on cost. A unique advantage of using *B. megaterium* as a source lies in the fact that the enzyme produced by it is an extracellular one that is relatively easy to separate and purify. On the other hand, the use of acetone-dried whole-cell enzyme may be economically attractive under certain circumstances. Acetone-dried whole-cell enzymes, like immobilized enzymes, can be reused in some cases.

Table 3. Activity of Penicillin Amidase from Bacterial Sources

Organism	Preparation	Activity (μ mol/min)	Spec.* Activity (units/mg)	Ref.
B. megaterium	Crude Broth	0.23	0.3	—
	70—fold purified	171.	21.	—
M. roseus	Whole Cell	1.1	0.1	—
E. coli (N. C. I. B. 8743)	Whole Cell	—	0.6	(21)
E. coli (ATCC 9637)	Cell Extract 200—fold purified	—	7.5	(20)

* For the whole-cell enzyme; micromoles/min/mg of cell, and for the pure enzyme; micromoles/min/mg of protein.

The enzyme activities listed in Table 3 are not necessarily the maximum attainable values. The methods by which the productivity of microbial enzymes can be increased have been the subject of many investigations, *e.g.*, Demain,[20,21] Pardee,[22] and Dean.[23] Some of the methods are genetic manipulation, control of environmental conditions, use of an inducer, and mode of inducer addition.

2) Reaction kinetics. *B. megaterium* was selected as the source of the enzyme for use in our studies of reaction kinetics. Both the soluble and immobilized

forms of penicillin amidase were prepared, and the reaction kinetics of the enzyme in a batch-reaction system were studied, with emphasis on the effect of the reaction products, 6-APA and PAA, on the enzymatic reaction. Our results show that 6-APA and PAA are noncompetitive and competitive inhibitors of penicillin amidase, respectively. This finding is consistent with those of Chiang and Bennett[19] and of Szentirmal.[15] It is interesting to note that the penicillin amidase obtained from *E. coli* has been reported to show substrate inhibitation.[24]

From our experimental results, we have determined the kinetic constants that are important in evaluating the performance of enzyme reactor systems. For both the soluble and immobilized forms of the enzyme, the values of K_m (Michaelis constant), K_{ip} (inhibition constant of 6-APA), and K_{ia} (inhibitation constant of PAA) have been determined, and are given in Table 4. Both inhibitation constants are considerably greater than the K_m value, and the value of K_{ia} is greater than that of K_{ip}.

Table 4. Kinetic Constants of Penicillin Amidase (*B. megaterium*)

	Soluble Enzyme	Insoluble Enzyme
K_M	4.5×10^{-3} M	6.0×10^{-3} M
K_{iP}	2.6×10^{-2} M	2.5×10^{-1} M
K_{iA}	0.45 M	0.62 M

The values of all three kinetic constants, K_m, K_{ip} and K_{ia}, are slightly greater for the immobilized enzyme than for the soluble enzyme. These differences may very well be due to mass transfer or a permeability barrier for the immobilized enzyme, as suggested by Lilly and Sharp.[25]

K_m values reported in the literature vary for the same enzyme from different sources, and also depend on the method of preparation. (See Table 5.) The K_m value of the immobilized enzyme reported by Self, Kay, and Lilly[26] is lower than that for the soluble form, contrary to our findings. They attributed this result to the electrostatic effect of the particular immobilized enzyme that they prepared.

Penicillin amidase has also been shown to be involved in the reversible coupling reaction of 6-APA and side chains, according to Rolinson *et al.*,[6] Batchelor *et al.*,[27] and Kaufmann, Bauer, and Offe.[28] They reported, however, that the hydrolytic reaction of the enzyme is predominant near pH 8.0. The enzyme obtained from *B. megaterium* did not show significant activity of the reversible coupling reaction of 6-APA and side chains.

Table 5. *Km* Values of Penicillin Amidase from Different Sources

Km (m*M*) (Soluble)	*Km* (m*M*) (immobilized)	Source Organism	Reference
4.5	6.0	*B. megaterium* (ATCC 14945)	
7.7	3.0	*E. coli* (ATCC 9637)	(20)
—	30.0 (Whole Cell)	*E. coli* (N.C.I.B. 8743A)	(21)
10.3		*S. lavendulse*	(25)
1.5		*E. coli*	(26)
2.5		*Fusarium senitectum*	(26)

3) Kinetic model of double inhibitation. Since two products of hydrolysis, 6-APA and PAA, are noncompetitive and competitive inhibitors of the enzyme, respectively, we have derived a kinetic model for the reaction of penicillin amidase that takes this combined inhibitory effect into consideration. The resulting rate expression for the penicillin amidase reaction is given in Equations (8) and (10), where,

v=reaction rate

k_2=reaction rate constant, as shown in Equation (2)

E_0=total enzyme concentration

S_0=initial substrate concentration, benzlpenicillin

X=conversion factor, as shown in Equation (9)

S=substrate concentration at time t

A=concentration of PAA

P=concentration of 6-APA

K_m=Michaelis-Menten constant

K_{ip}=inhibition constant of 6-APA

K_{ia}=inhibition constant of PAA.

The enzymatic reaction of the hydrolysis is shown in Fig. 1 and Equation (1). From batch experiments, the kinetic constants, k and K, were evaluated. The rate constants of individual reactions shown in Equations (2), (3), (4), (5), and (6) are represented by k_1, k_2, k_3, k_4, k_5, and k_6, and the equilibrium constants for substrate and products, as shown in Equation (7), are represented by K_m, K_{ia}, and K_{ip}.

In the formulation of the elementary steps of the reaction, Equation (3) describes the competitive inhibition by PAA, and Equations (4), (5), and (6) represent the noncompetitive inhibition by 6-APA.

Based on these elementary reactions, the rate expression Equation (8) was derived, and by substitution of more useful parameters given in Equation (9), the final form of the rate expression, shown as Equation (10), was derived. This Equation (10) is used in simulating the performance of the enzyme reactor.

4) Simulation of performance of an enzyme reactor system. Simulation studies based on a sound mathematical model enable one to predict the performance of an enzyme reactor system over a wide range of operating conditions and to determine the optimal process conditions. This approach reduces considerably the amount of experimental work required.

The enzyme reactor systems studied are shown in Fig. 2. An Amicon Model 402 type set-up was used as a single-stage continuous stirred tank reactor (CSTR) from which the product, as filtrate, can be

Kinetics of Double Inhibition

$$\underset{[S]}{\text{Benzyl penicillin}} \xrightarrow{\text{penicillin amidase}} \underset{[P]}{\text{6 APA}} + \underset{[A]}{\text{PAA}} \qquad [1]$$

$$[E]+[S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_2} [E]+[P]+[A] \qquad [2]$$

$$[E]+[A] \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} [EA] \qquad [3]$$

$$[E]+[P] \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} [EP] \qquad [4]$$

$$[EP]+[S] \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} [EPS] \qquad [5]$$

$$[ES]+[P] \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} [EPS] \qquad [6]$$

$$K_s=\frac{[E][S]}{[ES]},\quad K_{ia}=\frac{[E][A]}{[EA]},\quad K_{ip}=\frac{[E][P]}{[EP]} \qquad [7]$$

PAA; Competitive inhibition

6APA; Non-competitive inhibition

Kinetic Model

(combined doubleinhibition; competitive and noncompetitive)

$$v=\frac{k_2E_o}{1+\frac{K_m}{S}\left[1+\frac{A}{K_{ia}}\right]+\frac{P}{K_{ip}}\left[1+\frac{K_m}{S}\right]} \qquad [8]$$

$$X=\frac{S_o-S}{S_o},\quad S=S_o(1-X),\quad A=S_oX,\quad P=S_oX, \qquad [9]$$

$$v=\frac{k_2E_oS_o(1-X)K_{ia}K_{ip}}{[S_o(1-X)K_{ia}K_{ip}+K_mK_{ia}K_{ip}+K_mXS_oK_{ip}+S_o^2K_{ia}}$$

$$\overline{X(1-X)+S_oK_mK_{ia}]} \qquad [10]$$

removed continuously. An Amicon Model TCIS type set-up was used as part of a system that combined CSTR and an ultrafiltration unit.

The mathematical expressions describing these two systems are given in Equations (11), (12), and (13) where,

$\tau=V/Q$, space time of the enzyme reactor

Q= flow rate of substrate feed and also filtration rate

V=volume of the reactor.

If Equation (8) or (10) is substituted for v in Equation (11), this differential equation can be solved, together with the boundary conditions, by numerical methods. The fourth order Runge-Kutta method was used to solve this equation.

The boundary condition used was: $X=0$ when $t=0$. The equation was integrated from $t=0$ to $t=50$ hours. Except at very high concentrations of feed (S_o), the reactor systems considered reach a steadystate within this period.

For the combined system, the conversion that takes place inside the thin-channel separator was estimated by analyzing the ultrafiltration separator unit as a plug-flow reactor system. For this evaluation, the plug-flow reactor system was treated as a multistage CSTR system, and a numerical method was used to solve Equation (13), with $n=10$ stages.

The contribution made by the separator to the overall conversion of substrate to product was found to be negligible under the actual operating conditions, where the space time was less than 0.001 hr. Fig. 3

Reactor Kinetics

1. SINGLE-STAGE CSTR

$$\frac{dx}{dt}=\left(-\frac{1}{\tau}\right)x+\frac{1}{S_o}(v) \qquad (11)$$

$$\text{Productivity}=\left(\frac{S_ox}{\tau}\right)$$

2. CSTR and PLUG FLOW COMBINATION

$$\begin{cases}\dfrac{dx_1}{dt}=\left(-\dfrac{1}{\tau_1}\right)X_1+\dfrac{1}{S_o}(v_1)\\[2ex] \dfrac{dx_i}{dt}=\left(\dfrac{1}{\tau_i}\right)(X_{i-1}-X_i)+\dfrac{1}{S_o}(v_i)\end{cases} \qquad (12)$$

$$\text{Overall Productivity} \equiv \left\{\frac{S_o X_n}{\tau_1 + \sum_{i=2}^{n} \tau_i}\right\}$$

3. PLUG FLOW REACTOR (By the analysis of multi-staged reactor system)

$$\frac{dx_i}{dt} = \left(\frac{1}{\tau_i}\right)(X_{i-1} - X_i) + \frac{1}{S_o} v_i \qquad (13)$$

$$v_i = v(X_i)$$

$$\text{Overall Productivity} = \left\{\frac{S_o X_n}{\sum_{i=1}^{n} \tau_i}\right\}$$

shows this. For this reason, the combined system of CSTR and thin-channel ultrafiltration unit was considered as a single-stage CSTR system, for all practical purposes.

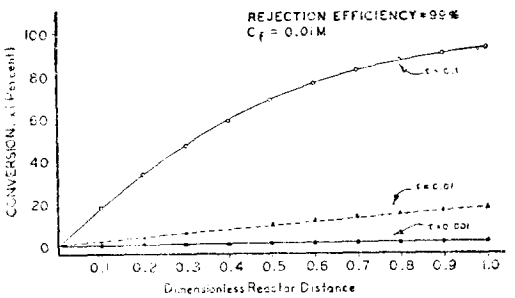

Fig. 3 Conversion effficiency, X, (the molar ratio of product, 6APA, to substrate, benzylpenicillin, in %) as a function of the distance of the plug flow reactor (thin channel ultra-filter system), The operating conditions shown are: three values of reactor space time, τ, one substrate feed concentration, C_F, and one rejection efficiency (the ratio of filtration rate to substrate feed rate),

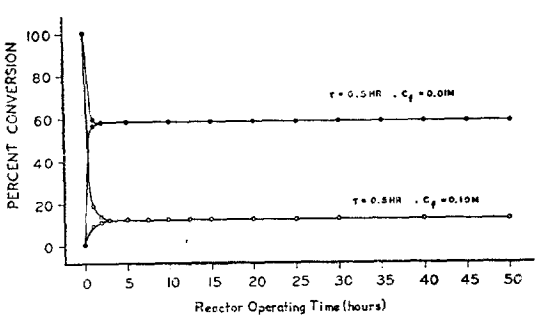

Fig. 4 Conversion efficiency as a function of the reactor operating time of the CSTR system under the given conditions of reactor space time and substrate feed concentrations, The transient period is relatively short irrespective of the initial conditions.

O'Neill, Lilly, and Rowe[29] reported the possible existence of multiple steady-states in enzyme reactor systems. In the case of penicillin amidase, it was found that only one steady-state was physically possible. This conclusion was based on the rsesults of analyses by solving for the steady-state solution, and the other by numerical integration of the differential equation, Equation (11). The steady-state equation for the CSTR system is given in Equation (14).

$$S_o^2 K_{ia} X^3 - (K_m K_{ip} S_o + K_m K_{ia} S_o + S_o^2 K_{ia} - S_o K_{ia} K_{ip}) X^2 - (S_o K_{ia} K_{ip} + K_m K_{ia} K_{ip} + V_{max} K_{ia} K_{ip}) X + V_{max} K_{ia} K_{ip} = 0 \qquad (14)$$

The roots of Equation (14) were found by using the Newton-Raphson method. Solution of the polynomial function, Equation (14), yields two positive roots and one negative root. However, only one root is of physical interest, since only this one root lies between zero and one, *i.e.*, $0 < X < 1.0$. This finding indicated clearly that there is only one steady-state solution of X (fractional conversion) that is physically meaningful. Fig. 4 shows that the final steady-state conversion value approaches a single value, irrespective of the initial concentration of substrate for a given feed substrate concentration.

Figs. 5 and 6 show the fractional conversion of benzylpenicillin to 6-APA as a function of space time and feed concentration. Although it was easy to predict that a greater conversion can be achieved with a lower feed concentration, and that the steady-state conversion increases with increasing space time, it was interesting to find that the immobilized enzyme reactor system gave higher conversion values than the soluble enzyme reactor for all feed concentrations, except for 0.01 M, at a given space time. Experimental results confirmed these predictons of the simulation studies. Under the operating conditions specified in Table 2 (*i.e.*, at space time of 7.7 hours), the fractional conversions are 64 and 83% for the soluble and immobilized enzymes, respectively. The results of simulation studies predicted that the corresponding conversions would be 60 and 80%, respectively, as shown in Figs. 5 and 6. This indicates that the experimental results were in good agree-

ment with the predictions obtained from the computer simulation.

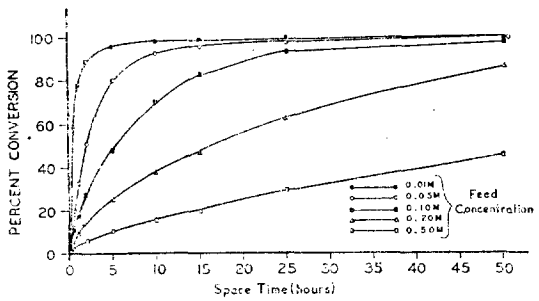

Fig. 5 The conversion efficiency in a continuous enzyme reactor containing soluble penicillin amidase as a function of reactor operating time (in terms of reactor space time) at varying substrate feed concentrations

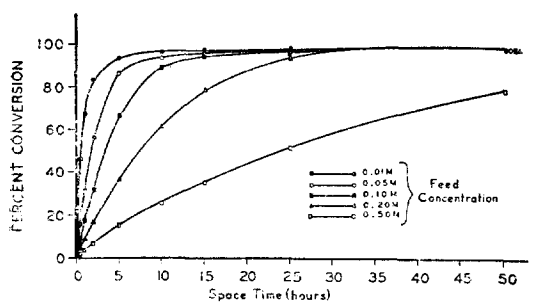

Fig. 6 The conversion efficiency in a continuous enzyme reactor containing immobilized penicillin amidase in suspension as a function of reactor operating time (in terms of reactor space time) at varying substrate feed concentrations

The difference in performance of the systems utilizing soluble and immobilized enzymes could be explained by a subtle interplay of various parameters involved in the reaction kinetics. As shown in Table 4, the K_{ip} value of the immobilized enzyme was greater than that of the soluble enzyme by one order of magnitude (0.25 and 0.026, respectively). Other kinetic parameters, K_m and K_{ia}, for the soluble and immobilized enzymes were of the same order of magnitude. The higher value of K_{ip} for the immobilized enzyme indicates that the inhibitory effect of the product on the immobilized enzyme was much less than that on the soluble enzyme at the same product concentration. Hence, there are, then, two opposing effects, namely the reaction rate, V_m, that is equivalent to the value of k_2E, and the effect of inhibition by the product. Generally, the maximum reaction rate, V_m, is higher and the K_m value is lower for the soluble enzyme than for the immobilized enzyme. These differences are normally reflected in higher conversion values for the soluble enzyme than for the immobilized enzyme provided that there is no inhibition by the product. In the case of penicillin amidase, however, the reaction rate is affected by product inhibition. The critical product concentration, above which the product inhibition becomes a predominant effect, is estimated to be 0.25 and 0.026 M for the immobilized and soluble enzyme, respectively.

Thus, it is important to recognize that we can take advantage of the inhibitory effect of a product by using a feed concentration of substrate above the critical value (*i.e.*, $S_o \geq 0.05$M).

At a low feed concentration of substrate (*i.e.*, $S_o = 0.01$ M), the product concentration is too low to cause the inhibition. When S_o is this low, the value of S_o is less than the values of K_{ip} or K_{ia} and the reaction rate is the predominant influence on the conversion. The reaction rate is highly dependent on substrate concentration when $S_o < K_m$.

Figs. 7 and 8 show productivity as a function of space time and substrate concentration in the feed, for reactors utilizing soluble and immobilized enzymes, respectively. The isoconversion lines are superimposed on these plots.

For any desired level of conversion of any substrate concentration of the feed, we will be able to determine the productivity as a function of space time. The isoconversion lines pass through maxima that correspond to the maximal productivity for a given conversion. Based on the information presented in Figs. 7 and 8, one can also determine the S_o (substrate feed concentration) and τ (the space time) that correspond to the maximal productivity for a desired conversion. The productivity, $S_o X/\tau$, is defined as moles of 6-APA produced per liter per hour. The

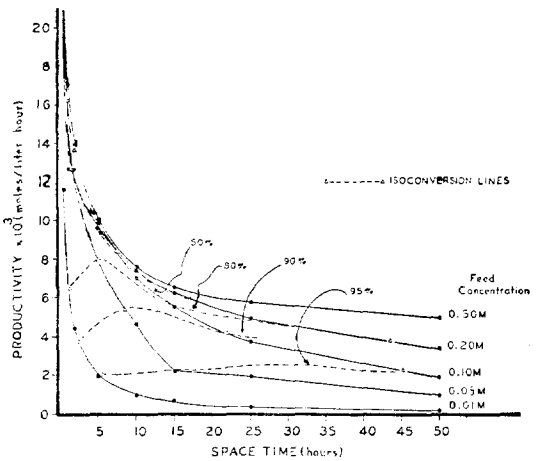

Fig. 7 The computer simulation of productivity (moles of 6APA produced per liter per hour) of the continuous enzyme reactor containing soluble penicillin amidase. The effect of reactor operating time on the productivity at varying substrate feed concentrations. The broken lines show the level of conversion efficiency (in %) that can be achieved under varying conditions of substrate feed concentration, reactor operating time, and the productivity

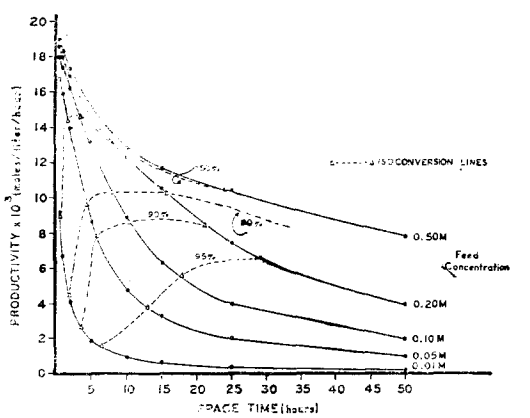

Fig. 8 The computer simulation of productivity (moles of 6 APA produced per liter per hour) of the continuous enzyme reactor containing immobilized penicillin amidase in suspension. The effect of reactor operating time on the productivity at varying substrate feed concentrations. The broken lines show the level of conversion efficiency (in %) that can be achieved under varying conditions of substrate feed concentration, reactor operating time, and the productivity

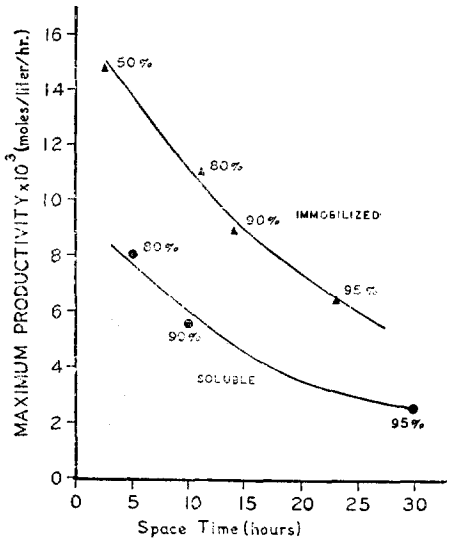

Fig. 9 Comparison of the performance of the enzyme reactor which contains soluble penicillin amidase and that which contains immobilized enzyme, based on the results of computer simulation shown in Figures 7 and 8

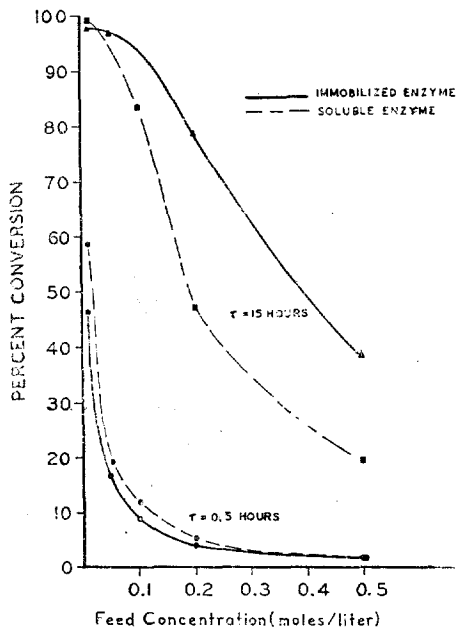

Fig. 10 Computer simulation of the effect of substrate feed concentrations on the conversion efficiency for both the soluble and immobilized enzyme reactor systems at varying reactor space time.

results shown in Figs. 7 and 8 represent, to a limited extent, an optimization of the process in terms of productivity and the key operating parameters.

Some important information obtained from Figs. 7 and 8 is summarized in Fig. 9. It shows the maxium productivity attainable under different operating conditions (*i. e.*, space time) and the desired level of conversion. We find that a reactor system utilizing immobilized enzyme gives higher productivity than one utilizing soluble enzyme, either at the same space time or at the same level of conversion.

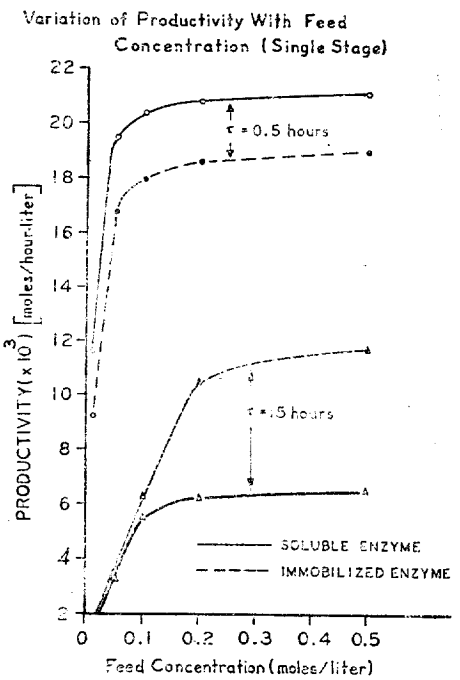

Fig. 11 Computer simulation of the effect of substrate feed concentrations on the productivity for both the soluble and immobilized enzyme reactor systems at varying reactor space time

We also studied the effect of substrate concentration in the feed (S_o) on conversion and productivity. The results of computer simulation are shown in Figs. 10 and 11. The fractional conversion falls rather dramatically as the S_o increases. When the space time is 0.5 hours, the conversion is too low to be practical, although the calculated productivity is higher than that for the space time of 15 hours.

When the space time is 15 hours, the value of S_o up to about 0.1 M seems practical in terms of conversion efficiency, although the conversion is too low to be practical beyond the value of S_o=0.2 M. The conversion for the soluble enzyme seems to fall more rapidly than does that for the immobilized enzyme.

At a 0.2 M feed concentration, the conversion attainable with immobilized enzyme is greater than 70%, whereas that attained with soluble enzyme is less than 50%.

From Fig. 11, it may be seen that productivity (for τ=15 hr) with the immobilized enzyme system is far greater than that with the soluble enzyme system, *e. g.*, at S_o=0.2 M, but the difference in productivity is negligible when S_o<0.1 M.

References

1. Price, K. E.; *Adv. Appl. Microbiol.* **11**, 17 (1969).
2. Claridge, C. A., Gourevitch, A., Lein, J.; *Nature* **187**, 237 (1960).
3. Huang, H. T., English, A. R., Seto, T. A., Shull, G. M., Sobin, B. A.; *J. Am. Chem. Soc.* **82**, 3790 (1960).
4. Kameda, Y., Kimura, Y., Toyoura, E., Omari, T.; *Nature* **191**, 1122 (1961).
5. Murao, S., Kishida, Y.; *J. Agric. Chem. Soc. Japan* **35**, 610 (1961).
6. Rolinson, G. N., Batchelor, F. R., Butterworth, J., Cameron-Wood, J., Cole, M., Eustace, G. C., Hart, M. V., Richards, M., Chain, E. B.; *Nature* **187**, 236 (1960).
7. Sakaguchi, K., Murao, S.; *J. Agric. Chem. Soc. Japan* **23**, 411 (1950).
8. Alburn, H. E., Grant, N. H., Clark, D. E.; *U. S. Patent* 3,032,473.
9. Weitnauer, G. A.; *U. S. Patent* 3,070,511.
10. Murao, S., Sakaguchi, K., Kono, K.; *U. S. Patent* 3,144,395.
11. Sjoberg, B., Nathorst-Westfelt, L., Ortengren, B.; *Acta Chem. Scand.* **21**, 547 (1967).
12. Gotovtseva, V. A., Gorchenko, G. L., Suvorov, N. N.; *Mikrobiologiya U. S. S. R.* **37**, 819(1968).
13. Chasin, L. A. Magasanik, B.; *J. Biol. Chem.* **243**, 5165 (1968).
14. Pruess, D. L., Johnson, M. J.; *J. Bacteriol.* **90**,

380 (1965).

15. Szentirmai, A.; *Antibiotics* (Herold, M., and Gabriel, Z.) 529, Butterworth Sci. Publ., London (1964).
16. Heuser, L. J., Chiang, C., Anderson, C. F.; *U. S. Patent* 3, 446, 705.
17. Batchelor, F. R., Chain, E. B., Hardy, T. L., Mansford, K. R. L., Rolinson, G. N.; *Proc. Roy. Soc. Ser. B.* **154**, 498 (1961).
18. Niedermayer, A. O.; *Anal. Chem.* **193** 938 (1964).
19. Chiang, C., Bennett, R. E.; *J. Bacteriol.* **93**, 302 (1967).
20. Demain, A. L.; *Developments in Ind. Microbiol.* **12**, 56 (1971).
21. Demain, A. L.; *Methods in Enzymology* (Colowick, S. P., Kaplan, N. O., Jakoby, W. B.) Vol. 22, 86, Academic Press, N. Y. (1971).
22. Pardee, A. B.; *Fermentation Advances*, (Perlman, D.) 3, Academic Press, N. Y. (1969).
23. Dean, A. C. R.; *5th International Continuous Culture Symposium*, in press, (1971).
24. Cole, M.; *Biochem. J.* **115**, 733 (1969).
25. Lilly, M. D., Sharp, A. K.; *Chem. Eng.*, London, **215**, CE12 (1968).
26. Self, D. A., Kay, G., Lilly, M. D.; *Biotechnol. Bioeng.* **11**, 337 (1969).
27. Batchelor, E. R., Chain, E. B., Richards, M., Rolinson, G. N.; *Proc. Roy. Soc. Ser. B.* **154**, 522 (1961).
28. Kaufmann, W., Bauer, K., Offe, H. A.; *Antimicrobial Agents Annual*, 1, Plenum Press, N. Y. (1960).
29. O'Neill, S. P., Lilly, M. D., Rowe, P. N.; *Chem. Eng. Sci.* **26**, 173 (1971).
30. Cole, M.; *Nature* **203**, 519 (1964).
31. Kaufmann, W., Bauer, K.; *Nature* **203**, 520 (1964).
32. Brandl, E.; *Hoppe-Seyl. Z.* **342**, 302 (1965).