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Immobilization of Whole-Cell Penicillin G Acylase by Entrapping Within Polymethacrylamide Beads

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ABSTRACT

Escherichia coli ATCC 11105 containing the periplasmic penicillin G acylase was entrapped within a copolymer of methacrylamide and *N,N'*-methylenebisacrylamide. A solution of monomer that was made up from methacrylamide and *N,N'*-methylenebisacrylamide dissolved in buffer was mixed with lyophilized cells and ammonium persulfate. This suspension was then pumped drop by drop into in soybean oil supplemented with 0.06% (v/v) 3-(dimethylamino)-propionitril. During submerging in the oil phase, the droplets were hardened and induced to polymerize within the droplets. Particles with a volume ranging from 0.013–0.017 mL per bead containing a biomass concentration up to 38.0 g/L were prepared. The optimal condition for the deacylation of penicillin G to 6-aminopencillanic acid (6-APA) catalyzed by the immobilized whole-cell penicillin G acylase was found to be 45°C and pH 8.0. Product inhibition of this enzyme by 6-APA could be eliminated by controlling pH value at 8 during the course of penicillin G hydrolysis using a pH-stat. Conversion determined by the pH-stat method were 0.3% higher than that by p-dimethylaminobenzaldehyde method. Cell concentration in the matrix was found to be an important factor influencing the maximum velocity and the specific activity retained in the matrix. A kinetic model, in which the mass transfer resistances as a result of external film mass transfer and pore diffusion were assumed to be negligible, could properly describe the hydrolysis of penicillin G by the cells entrapped within the polymethacylamide beads.

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Index Entries: Pencillin G acylase; polymethacrylamide; entrapment; immobilized whole-cell enzyme.

INTRODUCTION

Primary advantages of immobilized whole-cell enzymes are that they are easily prepared, can perform multistep, cofactor requiring bioconversion, and eliminate the costly process of enzyme isolation. Entrapment in polymeric matrices is a convinient method for the immobilization of cells. Mostly the three-dimensional network entrapping the cells is formed by the crosslinking of natural polymers like alginate or κ-carrageenan. The use of synthetic polymers such as polyacrylamide or polyurethane provides several advantages, for example, inertness to microbial attack, higher mechanical strength, chemical resistance, the diversity of using complexing buffer components and the option of the chemical composition and gel porosity that can be achieved by either copolymerization of very different available monomers or by chemical modification of performed polymers. Despite polymerization, conditions often result in a loss not only of enzyme activity, but also of cell viability caused by reactive intermediates or toxic monomers, acrylic polymers, especially the polyacrylamide, are the most synthetic matrices often employed for the immobilization of cells by entrapment as a result of their chemical structure with hydrophilic character *(1)*. Polyacrylamide matrices contain a hydrocarbon skeleton to which attached carboxamide side groups. These groups are chemically stable and resistant to hydrolysis in the pH range between 1 and 10. Cells are entrapped in polyacrylamide gel networks by a free-radical polymerization of acrylamide monomer in aqueous solution. The polymerization of acrylamide occurs in solution containing the cells, an initiator, and a crosslinking agent such as *N,N'*-methylenebisacrylamide. The resulting gel is then mechanically made into particles. Sato et al. *(2)* employed this method to obtain an immobilized *E. coli* ATCC 9637 containing penicillin G acylase. Prabhune et al. *(3)* immobilized the strain *E. coli* NCIM 2350 containing penicillin G acylase by entrapment in porous polyacrylamide beads coated with calcium alginate that was lately leached out after polymerization, based on the method of Pundle et al. *(4)*. Instead of acrylamide, we used here methacrylamide as the monomer for entrapment of cells by polymerization. In addition to carboxamide side groups on the hydrocarbon skeleton of the polymethacrylamide that provide the hydrophilic property, the methylene side groups can reduce the swelling of the polymeric beads in aqueous solution and then enhance their mechanical strength.

As one of the most important industrial enzyme, penicillin G acylase (penicillin G amidase, EC 3.5.1.11) catalyzes the formation of 6-aminopenicillanic acid (6-APA) from penicillin G. Besides being entrapped in polyacrylamide gel, the whole-cell penicillin G acylase (*E. coli* NCIM 2400) was immobilized by forming crosslinked aggregates that were reinforced with

silica and chitosan *(5)*. In this work, we immobilized the strain *E. coli* ATCC 11105 containing penicillin G acylase by entrapping in methacrylamide-based polymer beads using a simple procedure of polymerization in two-phase system. *E. coli* ATCC 11105 and its mutant derivatives produce higher enzyme activity and becomes the more commonly used strain for penicillin G acylase production *(6)*. Studies on the isolation and immobilization of penicillin G acylase from *E. coli* ATCC 11105 have been reported *(7,8)*. This paper addresses the influence of pH and cell concentration on the kinetics of the immobilized whole-cell associated penicillin G acylase.

MATERIALS AND METHODS

Growth of Cells

The strain *E. coli* ATCC 11105 was maintained on a slope of bacto-agar medium at 4°C. The stain from slope was growth on the medium 210 at 26°C for 10 h, with shaking (150 rpm). The medium 210 consisted of 2 g/L yeast extract, 2 g/L casein hydrolysate, 7 g/L K_2HPO_4, 3 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L sodium citrate and 2 g/L glucose. The inoculum was prepared from 0.1 mL of the ten-hour growth cells in 100 mL of cornsteep medium contained 20 g/L cornsteep liquor and 2 g/L phenylacetic acid (PAA). Incubation was shaken on a shaker (150 rpm) for 18 h at 26°C. Then, 15 mL of this culture were used to inoculate 300 mL of complex medium contained 20 g/L bacto-peptone, 5 g/L NaCl, and 2 g/L PAA in each 500-mL flask. Incubation was shaken again for 18 h at 26°C. Cells were harvested by centrifugation (10,000 rpm) at 4°C, lyophilized at –85°C and preserved at 4°C before immobilization.

Immobilization Method

The procedure of immobilization was based on a modification of the method employed by Nilsson et al. *(9)* for entrapping cells in polyacrylamide gel. The lyophilized cells (0.1–0.76 g) was dispersed in 10 mL of 50 m*M* phosphate buffer (pH 8) containing 0.05 g *N*-cetyl-*N,N,N*,-trimethylammonium bromide (CTAB) and shaken at 200 rpm for 30 min in a water-bath of 4°C. This cell suspension was then centrifuged (10,000 rpm), washed with phosphate buffer and centrifuged again. The supernatant was discarded and cells were dispersed in a monomer solution, prepared by adding 3 g methacrylamide, 2 g *N,N'*-methylenebisacrylamide and 5 mL *N,N'*-dimethyl formamide into 10 mL of 50 m*M* phosphate buffer. After addition of 0.4 g of an initiator ammonium persulfate to the cell suspension under stirring, the suspension was pumped drop by drop into a 5-L flat-bottom container of soybean oil by a peristalic pump with a flow-rate of 6 mL/min. The surface of the soybean oil was covered with 3 mL of 3-(dimethylamino)-propionitril (DMPAN). The droplets were formed as they contacted with DMPAN and stayed on the bottom of the container for

8 h. The polymerization of methacrylamide occurred within the droplets at room temperature. Finally, the cell-immobilized beads were collected and washed with distilled water and suspended in 50 m*M* phosphate buffer.

Activity Assays

One unit (U) of penicillin G acylase activity is defined as the amount of enzyme required to produce one micromole of 6-APA per minutes. Both the intact and immobilized cells were assayed for 6-APA production at 30°C for 30 min using a 50 m*M* solution of the potassium salt of penicillin G (Sigma Co., St. Louis, MO) in 0.05*M* phosphate buffer (pH 8). At the end of the enzymatic reaction, the immobilized cells were filtered and the filtrate was assayed for 6-APA content. Whereas the reaction catalyzed by intact cells was stopped by mixing with boiling water for 5 min. The 6-APA produced was estimated with p-dimethylaminobenzaldehyde *(10)*.

Time Course of Penicillin G Hydrolysis

Batch hydrolysis of penicillin G was carried out in flasks under rigorously stirring at 30°C. Each flask contained 200 beads of immobilized cells and 100 mL of 0.05*M* phosphate buffer (pH 8) containing potassium-penicillin G with an initial concentration of 5, 10, 30, or 50 m*M*. Samples were removed intermittently from the flasks and analyzed for liberated 6-APA using p-dimethylaminobenzaldehyde method *(10)*. Conversion is defined as the total moles of 6-APA in the reaction mixture divided by the total moles of penicillin salt initially present in the flask. Time course experiments of penicillin G hydrolysis also were carried out in a stirred flask equipped with an pH autotitrator FMS-201 (Cole Parmer, Niles, IL) controlling the pH of the reaction mixture at 8. In this case conversion is defined as the moles of PAA produced, monitored by measuring the amount of NaOH added to the reaction mixture by the autotitrator, divided by the total moles of penicillin G salt at the initial stage.

RESULTS AND DISCUSSION

Production of Penicillin G Acylase in *E. coli*

The production of penicillin G acylase followed the optimal procedure suggested by Robas et al. *(6)* using the cornsteep medium (CS medium) for the inoculum and the bactopeptone medium (complex medium) for the production, both supplemented with 2 g/L of PAA. Batch growth of *E. coli* ATCC 11105 in complex medium is shown in Fig. 1. The suggested harvesting time (18th h) falls in the latter stationary phase as can be seen from Fig. 1. It is believed that the enzyme activity is much stable in the stationary phase. For the production of penicillin G acylase, the presence of PAA in the culture medium is essential as proved by the pre-

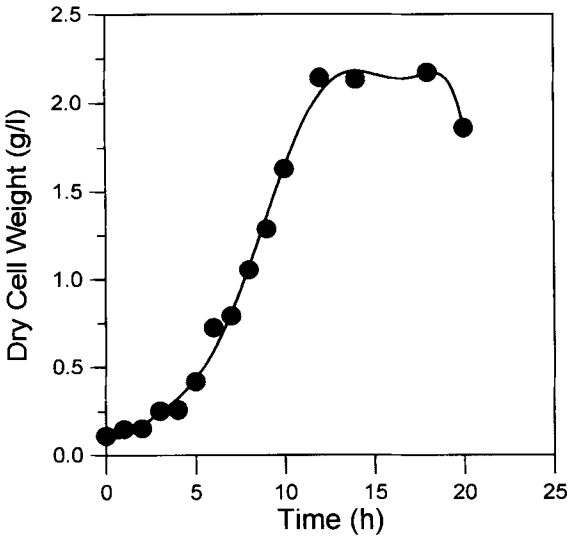

Fig. 1. Batch growth of *E. coli* ATCC 11105 in complex medium.

vious investigators *(6)*. As an inducer for production of penicillin G acylase, PAA happens to be the by-product of the hydrolysis of penicillin G to 6-APA and thus can be recovered for this usage. The penicillin G acylase activity of the intact cells falls in the range of 65–85 U/g cells, determined by the p-dimethylaminobenzaldehyde method.

Immobilized Whole-Cell Penicillin G Acylase Within Polymethacrylamide Beads

Beads of the prepared immobilized cell are not exactly of spherical. These cell-immobilized beads are button shaped with a diameter of ca. 3.5 mm and a height of ca. 2.5 mm. Since polymerization of the methacrylamide occurred at stationary state on the bottom of the container, the beads were of semispherical because of contact with the flat bottom. Particles with a volume ranging from 0.013–0.017 mL per beads containing a biomass concentration up to 38.0 g/L were obtained (Table 1). Using the intact cells with a specific activity of 79 U/(g cell), the immobilized cells possess specific activities from 12–35.4 U/(g cell), depending on the concentration of biomass entrapped within the matrix, as shown in Table 1. The retained percentages of activity were calculated to be from 15–45%. The retained portion of the penicillin G acylase after immobilization decreased with increasing cell concentration in the polymer matrix. This suggested that an increase in the cell population in the matrices could partially inhibit the active site of cell-associated enzymes, i.e., some enzymes were not accessible to the substrate. Table 1 also shows that the specific activity based on unit weight of matrices increased from 0.18–0.46 U/(g matrix) with cell concentration from 5.1–38.0 g/L. The prepared immobilized cells

Table 1
Results of Immobilized Cells

Cell concentration (g/L)[a]	Average volume of immobilized beads (mL/per bead)	Specific activity		Activity retained (%)[b]
		U/g matrix	U/g dry weight cells	
5.1	0.017	0.18	35.4	45
5.5	0.016	0.18	32.5	41
7.8	0.016	0.19	24.3	31
13.1	0.015	0.21	16.1	20
26.0	0.014	0.31	12.0	15
38.0	0.013	0.46	12.1	15

[a]Cell concentration (C_x) is defined as the ratio of cell dry weight to the total volume of the monomer solution.

[b]The specific activity of the intact cells, 79 U/g cells, was taken as 100%.

possessed higher values of penicillin G acylase activity than that (11.8 U/g cells against 4% w/v of K salt of penicillin G) reported by Prabhune et al. *(3)*. This level of penicillin G acylase activity is comparable to that (0.31 U/g matrix) of immobilized cells prepared by Sato et al. *(2)* though the cell concentration of their immobilized particles is much more higher (14% w/w). The experimental results suggested that polymethacrylamide should be considered to be of practical interest as an alternative matrix for entrapping the cells of *E. coli* containing penicillin G acylase. This polymeric beads take advantages of electroneutral and hydrophilic properties but not being subject to swell as much as polyacrylamide in aqueous solution. Since no charged groups on the matrix, the interactions between polymer molecules and entrapped cells or cell-associated enzymes are limited. Therefore, the kinetics of the immobilized enzyme could not be influenced by the matrix. The *E. coli* cells were treated with aqueous solution of the cationic detergent CTAB for 30 min before immobilization. This pretreatment was based on the previous suggestion *(11)* that the penicillin G acylase activity can be increased by permeabilization of the cells with CTAB.

Temperature Effect

The influence of temperature on the hydrolysis of penicillin G catalyzed by the intact and immobilized cells is shown in Fig. 2. All experiments were carried out under pH controlling at 8 by titration with 0.25*N* NaOH. It was observed the penicillin G acylase activity of the immobilized cells increased with temperature and reached to the maximum at 45°C and dropped suddenly at 50°C. Since before activity assays, the immobilized cells were preheated to the temperature of the substrate solution, the pre-

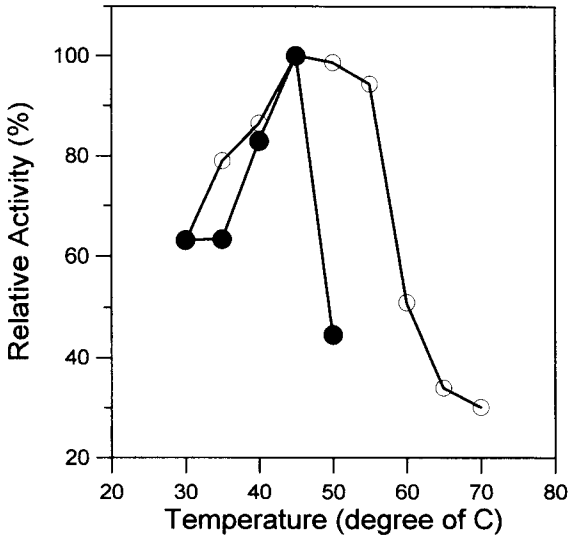

Fig. 2. Effect of temperature on rate of hydrolysis of penicillin G. (●) Immobilized cells; (○) intact cells. The maximum specific activity measured (intact cells, 123 U/g cell, immobilized cells, 0.214 U/g matrix) was taken as 100%.

heating process on the reused immobilized cells could contribute a loss of some activity at higher temperature. The optimal temperature was 45°C for both intact and immobilized cells. Whereas the optimal pH was ca. 8 for both intact and immobilized cells, which agrees with that for penicillin G acylase isolated from *E. coli* ATCC 11105 reported by Erarslan and Guray *(7)*. Since this polymer is electroneutral and dose not give rise to charge on interactions with cells, cell-bound enzymes, or proton in the substrate solution, there was no obvious shift in the pH optimum of the activity of penicillin G acylase. According to the literature, this enzyme is much more stable at 30°C than that at its optimal temperature for activity. The batch experiments for time course of penicillin G hydrolysis were thus carried out at 30°C in this work. The prepared immobilized cells retained 85% of their original activity after incubation for penicillin G hydrolysis six times (each 6 h) and preservation at 4°C for 30 d.

Influence of pH on Penicillin G Hydrolysis

The decrease of pH in the solution of the reaction mixture during penicillin G hydrolysis was a result of the deprotonization of PAA produced. Figure 3 shows that the rate of penicillin G hydrolysis was significantly affected by the production of proton in the solution and the retardation of the reaction because of the decline of pH could be improved by controlling the pH of the reaction solution. Based on 6-h reactions, the average hydrolysis rate catalyzed by immobilized cells without pH adjustment is 12% lower than that with pH adjustment during the course of

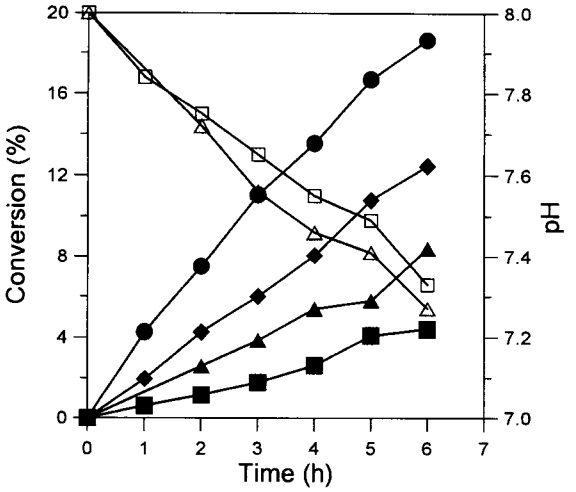

Fig. 3. Influence of pH on the hydrolysis of penicillin G. (●, ▲, Δ) Intact cells with total activity of 1.08 U; (◆, ■, □) immobilized cells with total activity of 0.51 U and C_x = 38.0 g/L. (●, ◆) Time course of penicillin G hydrolysis in flasks equipped with pH control at pH 8; (▲, ■) without pH control. The pH decrease in the presence of intact cells (Δ) and immobilized cells (□). Temperature, 30°C; the initial substrate concentration, 50 m*M*. Conversions was calculated based on the production of 6-APA.

hydrolysis. On the other hand, it was 10% lower for intact cells. It was evident that a decrease of pH in the solution reduced the enzyme activity and consequently slowed down the increase of conversion. Probable change of enzyme conformation caused by the decrease of pH in the solution contributed the reduction in the maximum reaction rates of penicillin G hydrolysis catalyzed by both intact and immobilized cells.

Comparison of Two Analytical Methods

Two methods, p-dimethylaminobenzaldehyde and pH-stat, were employed for the determination of production formation. The assay of measuring the amount of 6-APA depends on the reaction of free amino group of 6-APA with p-dimethylaminobenzaldehyde to form a coloured Schiff's base. The amount of PAA produced by the reaction catalyzed by penicillin G acylase was determined by measuring the amount of base added to the solution. Plot of the consumption of 0.25N NaOH vs the concentration of PAA present in the buffered solution (50 mL) is a straight line emerging from the origin with a sploe of 0.2. Results from these two methods are compared in Fig. 4. The pH-stat method could generate a small error as a result of dilution by the addition of alkaline solution to the reaction mixture. It contributes a 4.5% of volume increase in the course of penicillin G hydrolysis for 6 h with an initial concentration of substrate 30 m*M* in 100 mL of reaction solution. On the contrary, the method of p-dimethylaminobenzaldehyde resulted in an error as a result of the reduction of

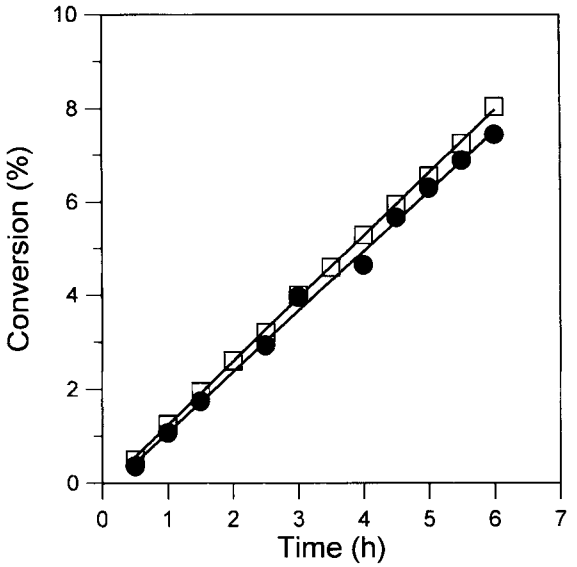

Fig. 4. Conversions of penicillin G catalyzed by immobilized cell (200 beads, C_x = 13.1 g/L) determined by pH-stat method (□) and p-dimethylaminobenzaldehyde method (●). The experiment was carried out at 30°C, in 100 mL of substrate (50 m*M*) at pH 8.

volume by sampling. For each time course experiment, ten samples summing up 5 mL of the reaction volume were taken. These two methods differed 0.3% in average based on the calculation of conversion. The figures obtained by the method of pH-stat were always higher than that from the method of p-dimethylaminobenzaldehyde. However, the pH-stat has an advantage of combining the determination of the product from the reaction catalyzed by penicillin G acylase as well as the maintenance of the solution pH at a constant value. Furthermore, the dilution effect as a result of the addition of base by autotitrator can be reduced to an insignificant level by using a higher concentration of NaOH.

Kinetics of Penicillin G Hydrolysis by Immobilized Whole-Cell Penicillin G Acylase

The kinetics of penicillin G hydrolyzed by penicillin G acylase is well documented *(12,13)*. The rate equation can be written as

$$v = \frac{V_m S}{S + K_m + \frac{K_m}{K_{ip}} P + \frac{K_m}{K_{iq}} Q + \frac{K_m}{K_{ip} K_{iq}} PQ + \frac{S}{K_{ip}} P} \quad (1)$$

where K_m is the Michaelis-Menten constant, K_{ip} and K_{iq} are the inhibition constants for P, phenylacetic acid, and Q, 6-aminopenicillanic acid, respectively, and V_m is the maximum reaction rate (mol/h g of cell). Consider W_c

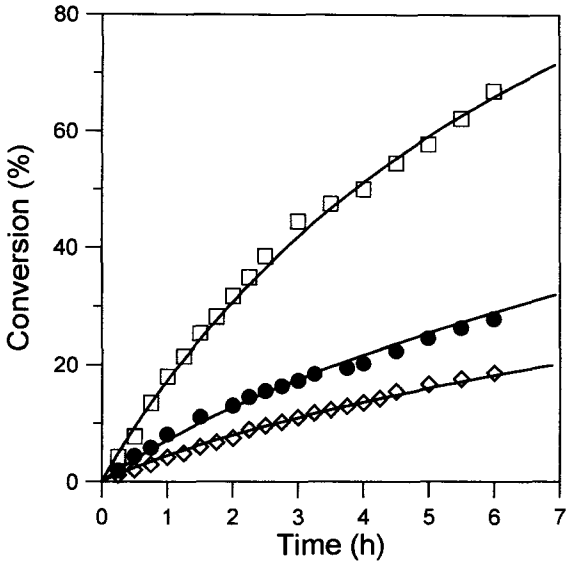

Fig. 5. Hydrolysis of penicillin G by intact cells at 30°C and pH 8. The initial substrate concentrations were 10 (□), 30 (●), and 50 m*M* (◇), respectively. Conversions were calculated based on the production of PAA (pH-stat method). Solid lines correspond to the best-fit using Eq 4 with the following kinetic parameters: V_m = 3.49 mmol/h/g dry weight cell, K_m = 4.04 m*m* K_{ip} = 6.31 m*M*, and $K_{iq} = \infty$, V_l = 100 mL and W_c = 81.4 mg.

grams of dry weight cell is present in V_l of penicillin G (K salt) solution with an initial concentration of S_0. The mass balance for substrate and products in the solution can be expressed respectively as

$$-\frac{dS}{dt} = \frac{W_c}{V_l} v \tag{2}$$

and

$$\frac{dP}{dt} = \frac{dQ}{dt} = -\frac{dS}{dt} \tag{3}$$

Applying the initial conditions: t = 0, P = Q = 0, S = S_0, and the definition of conversion $X = (S_0 - S)/S_0 = P/S_0$, the variation of conversion with time can be obtained as

$$\frac{W_c}{V_l} V_m t = S_0 X - K_m \ln(1-X)[1 + \frac{S_0}{K_{ip}} + \frac{S_0}{K_{iq}} + \frac{S_0^2}{K_{ip}K_{iq}}] + \frac{S_0^2 X^2}{2K_{ip}}$$
$$-K_m S_0 X(\frac{1}{K_{ip}} + \frac{1}{K_{iq}} + \frac{S_0}{K_{ip}K_{iq}} + \frac{S_0 X}{2K_{ip}K_{iq}}) \tag{4}$$

Matching the experimental results from the hydrolysis of penicillin G catalyzed by intact cells with the predicted time course described by equa-

tion 4 (Fig. 5), the kinetic parameters were obtained as: V_m = 3.49 mmol/h (g dry weight cell), K_m = 4.04 m*M*, K_{ip} = 6.31 m*M*, and $K_{iq} = \infty$. The K_m value approached to that (3.18 m*M*) of penicillin G acylase isolated from a mutant strain of *E. Coli* ATCC 11105 *(8)*. The infinite value of K_{iq} stands for a release of product (6-APA) inhibition. Inhibition of penicillin G acylase by 6-APA has been showed to be noncompetitive *(10)*. The release of inhibition by 6-APA suggested that the binding of 6-APA to penicillin G acylase (not at the active sites) is enhancing by the production of proton, i.e., lower pH value of the reaction mixture. As reported by Balasingham et al. *(10)*, K_{iq} varies markedly with pH. When the pH of the reaction solution remained constant at 7, the inhibition by 6-APA disappeared.

Based on the ideal of insignificant external film-mass-transfer resistance and internal diffusional resistance through the matrix pore of the polymethacrylamide beads harboring the cells, the mass balance for substrate can be written as

$$-\frac{dS}{dt} = \frac{V_p}{V_l + V_p} C_x \nu \tag{5}$$

where V_p is the total volume of the beads. The assumption of negligible effects of pore diffusion and external film-mass-transfer is supported by the observation that the concentrations of both substrate and products suddenly reached to their equilibrium levels in diffusion-in experiments. In determining rate parameters for kinetics of enzymatic reaction, the experimental data are typically obtained from initial-rate experiments. An expression based on equation 5 for initial-rate data resulting from hydrolysis experiments using immobilized whole-cell enzymes can be written as (Hanes-Woolf plot)

$$-\frac{V_p}{V_l + V_p} \frac{S_0}{dS/dt\big|_{t=0}} = \frac{S_0}{C_x V_m} + \frac{K_m}{C_x V_m} \tag{6}$$

The values of maximum velocity were thus determined from the slopes of the Hanes-Woolf plot to be 2.5, 1.4, and 1.3 mmol/h/(g cells) for the beads with cell concentrations of 5.1, 26.0, and 38.0 g/L, respectively. V_m decreased with increase of cell concentration was observed caused by the loss of enzyme activity. For immobilized cells system, the time course of penicillin G hydrolysis represented by conversion-time relationship is the same as equation 4 except the left hand side, i.e.,

$$\frac{V_p}{V_l + V_p} C_x V_m t = S_0 X - K_m \ln(1 - X)\left[1 + \frac{S_0}{K_{ip}} + \frac{S_0}{K_{iq}} + \frac{S_0^2}{K_{ip} K_{iq}}\right] + \frac{S_0^2 X^2}{2K_{ip}} - K_m S_0 X\left(\frac{1}{K_{ip}} + \frac{1}{K_{iq}} + \frac{S_0}{K_{ip} K_{iq}} + \frac{S_0 X}{2K_{ip} K_{iq}}\right) \tag{7}$$

It is noted that the volume of particle V_p cannot be ignored and the actual initial substrate S_0 related to substrate concentration prepared in V_l of

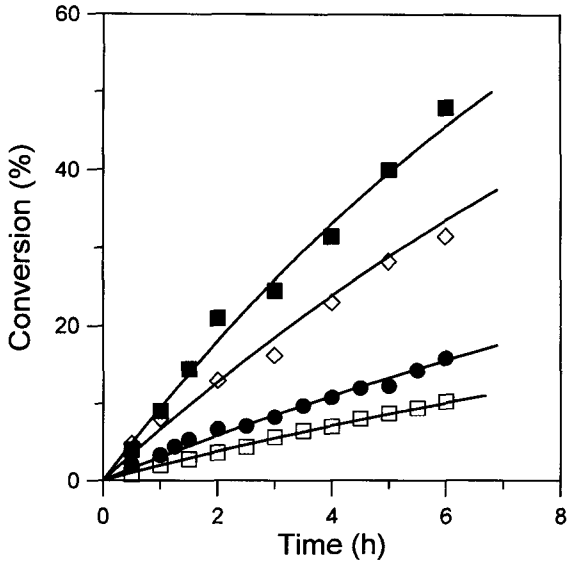

Fig. 6. The predicted and experimental time courses of penicillin G hydrolysis by immobilized cells with a cell concentration of 26.0 g/L. Experiments were carried out at 30°C and pH 8 using the pH-stat with initial substrate concentrations of 5 (■), 10 (◇), 30 (●), and 50 m*M* (□). Conversions were calculated based on the production of PAA. Solid lines correspond to the predicted time courses using equation 7 with the following parameters: K_m = 4.04 m*M*, K_{ip} = 6.31 m*M*, and $K_{iq} = \infty$, V_m = 1.4 mmol/h/(g dry weight cell), V_p = 2.72 mL, V_l = 100 mL, and C_x = 26.0 g/L.

buffer by a factor $V_l/(V_l + V_p)$. Figure 6 demonstrates the predicted (by Equation 7) and experimental time course of penicillin G hydrolysis catalyzed by polymethacrylamide beads harboring cells with a concentration of 26.0 g/L. The well matching between the predicted and experimental suggested that the assumption of negligible diffusion effects is acceptable.

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NOMENCLATURE

C_x dry biomass concentration within the matrix (g/L)
K_{ip} inhibition constant for phenylacetic acid *(M)*
K_{iq} inhibition constant for 6-aminopenicillanic acid *(M)*
K_m Michaelis constant *(M)*
P phenylacetic acid concentration in the solution *(M)*

Q 6-aminopenicillanic acid concentration in the solution *(M)*
S penicillin G concentration in the solution *(M)*
S_0 initial penicillin G concentration in the solution *(M)*
t time (h)
v velocity of the reaction (mol h^{-1} g^{-1})
V_l volume of the solution (mL)
V_m maximum rate of penicillin G hydrolysis per unit dry weight of cell (mol h^{-1} g^{-1})
V_p total bead volume (mL)
W_c total dry weight of cell (g)
X conversion

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