PRODUCTION OF PENICILLIN IN A FLUIDIZED-BED BIOREACTOR USING URETHANE FOAMS AS CARRIERS

TETSUO KOBAYASHI AND KENJI TACHI
Technical Development Div., SHINKO PANTEC Co., Ltd., Kobe 651
TERUYUKI NAGAMUNE AND ISAO ENDO
Chemical Engineering Laboratory, The Institute of Physical and Chemical Research, Wako 351-01

Key Words: Biochemical Engineering, Penicillium Chrysogenum, Penicillin Production, Immobilized Cell, Urethane Foam, Batch Operation, Repeated Batch Operation, Fluidized Bed, Volumetric Mass Transfer Coefficient

By using urethane foams as immobilizing carriers for *Penicillium chrysogenum*, the effect of apparent volumetric mass transfer coefficient of oxygen k_La on penicillin production in a fluidized-bed bioreactor (FBR) was investigated. The k_La value was measured by varying the amount of urethane foam carriers added as well as aeration rate, suspended cell mass concentration and antifoam reagent concentration in a model cultivation system. High production of penicillin by batch operation was obtained in the operational region where the volumetric ratio of carriers in the working volume of FBR was 0.3 to 0.4 and gas superficial velocity was 0.03 to 0.05 $m \cdot s^{-1}$. Under these fluidizing operational conditions, a repeated batch operation was performed. The adhered cells in the carriers could be used repeatedly for semicontinuous production of penicillin, and a penicillin yield of about twice the quantity of lactose utilized could be obtained.

Introduction

It is widely known that in a conventional submerged culture molds and actinomycetes show pellet growth or pulpy growth according to a slight change in inoculum size of spore.[8,9] This growth morphology of cells affects the mass transfer of oxygen and/or that of substrate in the submerged culture system, and thus the production rate of antibiotics.[2,6,7] The separation of the metabolic product from the culture broth as well as the monitoring and controlling of the culture system was also influenced by the morphology of cells.[4]

By using soft, porous urethane foams as immobilizing carriers, a novel cultivation method for mycelia was studied in order to increase the productivity of antibiotics.[3] The effects of urethane foams on the growth and penicillin production characteristics of *Penicillium chrysogenum* were studied in shaking-flask culture systems.[4] It was found that *P. chrysogenum* crept into the carrier and then formed loose biofilm of 0.4 to 0.5×10^{-3} m thickness inside the carriers. This growth feature was effective in reducing the viscosity of the culture broth, thus improving the mass transfer rates of substrate and of oxygen. With this method, penicillin production could be six times that by conventional cultivation methods.

* Received June 9, 1989. Correspondence concerning this article should be addressed to T. Kobayashi.

The first purpose of this study was to elucidate the effect of apparent volumetric mass transfer coefficient k_La on the production level of penicillin in a fluidized-bed bioreactor (FBR) system to which urethane foams were added. The second was to investigate the possibility of a repeated batch culture operation for semicontinuous penicillin production by using the adhering grown cells in the carriers.

1. Expermental

1.1 Organism and culture medium

All experiments were performed using *Penicillium chrysogenum*, JCM 2056 (sp. Q176). It was maintained in a potato dextrose agar slant (39 $kg \cdot m^{-3}$ distilled water (Difco)) at a temperature of 5 °C. The basal medium composition of the culture medium used is shown in **Table 1**.

1.2 Urethane foam

Several kinds of urethane foams which are available for the carriers of *P. chrysogenum* have been examined.

Table 1. Basal medium composition

Composition	Amount
Lactose	40.0 g
Corn-steep liquor	20.0 g
$NaNO_3$	3.0 g
KH_2PO_4	0.5 g
$MgSO_4$	0.25 g
Distilled water	1.0 dm^3

Among them, a gelatin-coated urethane foam (sold as "artificial soil" by Nitto Denko Co., Ltd.) was proved to be best for our purpose. Because this urethane foam was modified into hydrophilic material, cells can anchor on it easily and stably. This carrier was used in the experiments by cutting the urethane foam sheet into cubes of two sizes, with side lengths l_0 of 2.5 and 5.0×10^{-3} m. The porous size of the foam is 60 to 120 PPI (pores/inch), its porosity is 0.94 to 0.96 and the apparent density ρ_0 is 90 to 110 $kg \cdot m^{-3}$.

1.3 Culture conditions and operations

FBRs of two sizes, $2.0 \times 10^{-3} m^3$ and $10.0 \times 10^{-3} m^3$ (0.075 m and 0.14 m inside diameter respectively) were used in these experiments. They are shown in **Fig. 1.** These FBRs were charged with $0.8 \times 10^{-3} m^3$ of culture broth and $8.0 \times 10^{-3} m^3$ of culture broth respectively. The inoculum size of spores in the basal medium were adjusted to 1×10^9 to 3.0×10^9 to 3.0×10^9 spores/m^3 and the initial pH was adjusted to 4. The pH of fermentation broth was maintained below 6.0. A thermostat was employed to maintain the temperature of culture broth at 25°C. Aeration rates were controlled so as to maintain dissolved oxygen concentration at 40% of saturation or higher. The amount of urethane foams added into the culture broth, W, was varied from 10 to 30 $kg \cdot m^{-3}$. The initial concentration of antifoam reagent (Silicone KM-70, Shin-Etsu Chemical Co., Ltd.) was adjusted to $0.8 \times 10^{-3} m^3 \cdot m^{-3}$. Repeated batch cultivation of penicillin in the initial batch culture reached maximum, the culture broth was withdrawn through a perforated plate at the bottom of the FBR and fresh medium was then fed to the reactor, where adhering grown cells remained in the carriers. To estimate the penicillin production activity of each spore stored, control cultivation in shaking-flasks ($0.3 \times 10^{-3} m^3$ with 30 $kg \cdot m^{-3}$ of urethane foams) under the same cultivation condition was done in parallel with the initial batch cultivation in the FBR.

1.4 Measurement

Penicillin concentration P was determined by both biological assay and HPLC (column: Shenshupak C8-1251S; room temperature; carrier: CH_3CN 25%, 0.01M NaH_2PO_4 75%; flow rate: $4.8 \times 10^{-5} m^3 \cdot h^{-1}$; volume of culture broth injected: $10^{-8} m^3$; detector: HITACHI 635). Lactose concentration L was analyzed by HPLC (column: IonpakKS801; temperature: 60°C; carrier: distilled water; flow rate: $4.8 \times 10^{-5} m^3 \cdot h^{-1}$; volume of culture broth injected: $10^{-8} m^3$; detector: RI monitor SE-31). The leaked cell mass concentration X_l from carriers and the adhered cell mass concentration X_a were measured as follows. The urethane foam carriers with adhering cells were separated from culture broth, then the culture broth was filtrated by paper filter (No. 2, Toyo Roshi Co. Ltd.). The paper filter was washed several times with distilled water and dried at 105°C for 48 h, and then weighed. X_l was determined from the difference in weight between the paper filter with attached dried cells and one without. X_a was measured from the difference in weight between urethane foams with adhering dried cells and ones without.

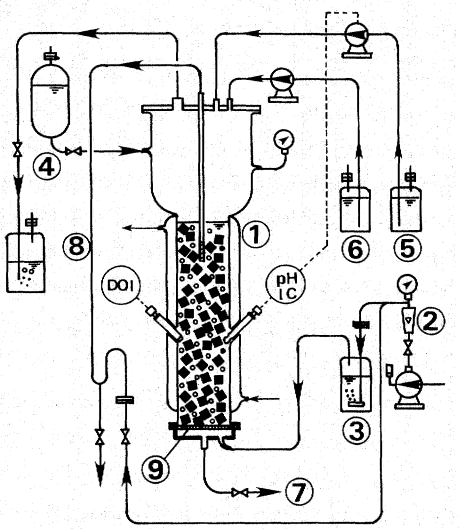

Fig. 1. Flow diagram of fluidized-bed bioreactor using urethane foam particles
(1) fluidized-bed bioreactor; (2) gas flow meter; (3) humidifier; (4) medium storage tank; (5) HCl storage bottle; (6) antifoam storage bottle; (7) drain pipe; (8) sampling pipe; (9) perforated plate

The apparent volumetric mass transfer coefficient of oxygen k_La was measured using the dynamic gassing-out method[10]. By varying the size of urethane foam carriers l_0 and the additional amount of them W, the effect of addition of the carriers on k_La in the FBR was studied. To evaluate both the effects of leaked cell mass concentration in culture broth and antifoam reagent concentration on k_La in the FBR system with urethane foam, the K_La values in a suspended-cells system and in a system to which antifoam reagent was added with the cells were also measured.

2. Results and Discussion

2.1 The method of calculating k_La in the FBR system

The production of penicillin is known to be greatly influenced by oxygen transfer in the culture system, because *P. chrysognum* is a strict aerobic microorganism. To evaluate the production efficiency of the FBR system, the apparent volumetric mass tranfer coefficient of oxygen k_La has been adopted as a criterion. Thus the method of calculating k_La in the FBR system has been studied to clarify the relation between the amount of penicillin produced and the value of k_La.

Firstly, the K_La in the FBR system was expected to be influenced by the additional amount of urethane

foam carriers (we expressed them in terms of the urethane foam holdup ε_{bp} as the volumetric ratio of urethane foam carriers to the total fluidized volume of FBR), their size l_0, the inside diameter of the FBR and gas superficial velocity U_G. Furthermore, k_La during fermentation was reported to be influenced by the concentration of leaked cell mass X_l in the culture broth and the concentration of antifoam reagent C_A.[1)]

Fixing the side length of cube l_0 of carriers and the inside diameter of the FBR at constant values in the following discussion, effects of the other four factors on k_La in the FBR system can be investigated. Namely, let k_La in each system be expressed as follows:

$k_La(U_G, \varepsilon_{bp}, 0, 0)$: k_La in a system to which only urethane foams are added.

$k_La(U_G, \varepsilon_{bp}, X_l, 0)$: k_La in a system containing both urethane forms and suspended cells.

$k_La(U_G, \varepsilon_{bp}, X_l, C_A)$: k_La in a system containing urethane foams, suspended cells and antifoam reagent. The dimensionless coefficients α, β, and γ are defined as follows:

$$\alpha(U_G, \varepsilon_{bp}) = k_La(U_G, \varepsilon_{bp}, 0, 0)/k_La(U_G, 0, 0, 0) \quad (1)$$

$$\beta(U_G, \varepsilon_{bp}, X_l) = k_La(U_G, \varepsilon_{bp}, X_l, 0)/k_La(U_G, \varepsilon_{bp}, 0, 0) \quad (2)$$

$$\gamma(U_G, \varepsilon_{bp}, X_l, C_A) = k_La(U_G, \varepsilon_{bp}, X_l, C_A)/k_La(U_G, \varepsilon_{bp}, X_l, 0) \quad (3)$$

Here, $k_La(U_G, 0, 0, 0)$ means the k_La in a gas-liquid system (bubble column).

By using these coefficients and $k_La(U_G, 0, 0, 0)$, the apparent volumetric mass transfer coefficient of oxygen $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ in the FBR system can be expressed as in Eq. (4).

$$k_La(U_G, \varepsilon_{bp}, X_l, C_A) = \alpha \cdot \beta \cdot \gamma \cdot k_la(U_G, 0, 0, 0) \quad (4)$$

The value of $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ can be calculated by determining these coefficients. As examples, experimental results obtained by using urethane foam cubes with side length of 5×10^{-3} m and FBR with 0.075 m inside diameter are presented below.

According to the increments in U_G and ε_{bp}, four flow patterns were observed as shown in **Fig. 2.** It was also found that k_La increased in proportion to U_G but decreased with increament in ε_{bp}. On the basis of these experiments, a contour map of k_La against ε_{bp} and U_G was drawn as shown in Fig. 2. It is clear from this figure that there exist transition points where k_La decreased sharply against a certain ε_{bp} value even thouth U_G increased. These deteriorations of k_La are considered to be caused by the drastic changes in the flow pattern from turbulent-circulation flow to slugging flow.

It is clear from **Fig. 3** that α decreased according to the increments in ε_{bp} and U_G. Many large bubbles could be observed visually at higher values of ε_{bp}. The k_La value has been investigated in three-phase fluidized

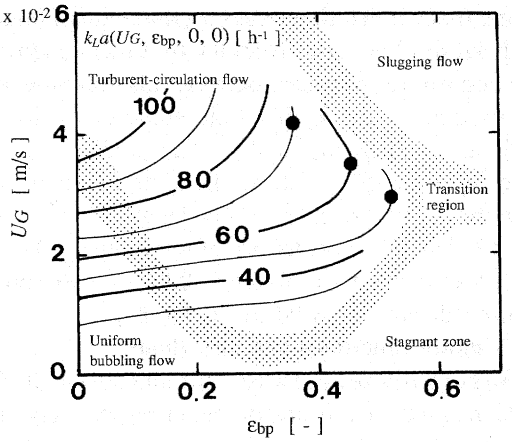

Fig. 2. Effects of U_G and ε_{bp} on k_La (U_G, ε_{bp}, 0, 0)
Diameter of FBR: 0.075 m, l_0: 0.005 m

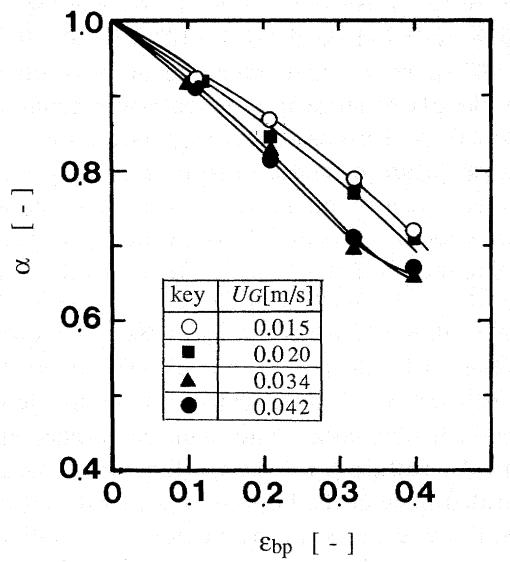

Fig. 3. Effect of urethane foams addition on apparent volumetric mass transfer coefficient of oxygen
Diameter of FBR: 0.075 m, l_0: 0.005 m

beds by using denser (glass and metal) particles.[5)] It was reported that k_La in fluidized beds of denser particles was higher than that in a bubble column and increased with increases in particle diameter and density. It was suggested that denser particles broke gas bubbles more easily than smaller and lighter ones did, (resulting in a large gas-liquid interfacial area. However, the density of urethane foam carriers soaked in culture broth was from 1005 to 1030 $kg \cdot m^{-3}$, which was almost the same as that of the culture broth. It seemed that urethane foam carriers promoted coalescence of bubbles and this decreased the interfacial area of bubbles. Hence the fact that k_La was lower in the FBR than in the bubble column was considered to be characteristic of the substantially low density of urethane foam carriers.

Both β and γ were examined by changing U_G from 0.01 to 0.045 $m \cdot s^{-1}$. In **Fig. 4**, β values are observed

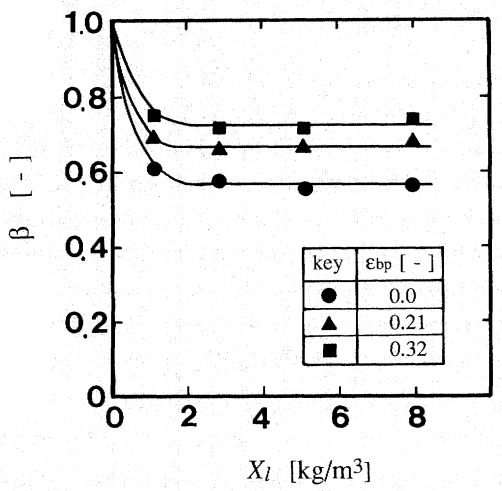

Fig. 4. Effect of leaked cell mass in the culture broth on apparent volumetric mass tranfer coefficient of oxygen
Diameter of FBR: 0.075 m, l_0: 0.005 m, U_G: 0.01–0.045 m.s^{-1}

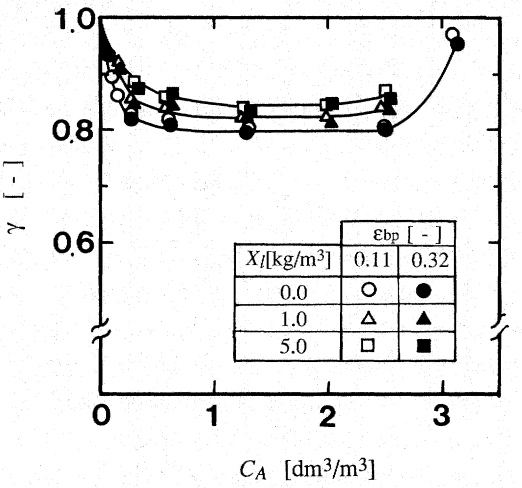

Fig. 5. Effect of the antifoam reagent concentration on the the apparent volumetric mass transfer coefficient of oxygen
Diameter of FBR: 0.075 m, l_0: 0.005 m, U_G: 0.01–0.045 m.s^{-1}

to decrease according to the increment in X_l but to remain almost constant above the X_l value of 2 kg·m^{-3} and have a tendency to increase with increasing of ε_{bp}, irrespective of U_G. It is seen in **Fig. 5** that γ showed an almost constant value of 0.8 to 0.85 according to the increase of X_l within the range of C_A from 0.8 to 2.0×10^{-3} m^3·m^{-3}, which is almost equal to the range of C_A in the FBR during the cultivation. γ was unrelated to either U_G or ε_{bp}.

Thus, α, β and γ were expressed as functions of (ε_{bp} and U_G), (ε_{bp} and X_l) and (X_l and C_A) respectively.

2.2 Production level of penicillin in the FBR system

By varying the additional amount of urethane foam carriers and their size l_0, the production of penicillin in a batch operation was first tested in order to clarify the operational conditions of the FBR system. The time course of production level of penicillin in the FBR was almost the same as that in a shaking-flask, and the maximum amount of penicillin was attained around the 8th day after cultivation was started. The relation between the relative concentration of penicillin produced P_m^* and $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ is shown in **Fig. 6**. Here, P_m^* was normalized by the maximum concentration of penicillin obtained in the control fermentation with urethane foam carriers by using the shaking-flask.

It is clear from Fig. 6 that P_m^* increased according to the increment in $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$, irrespective of the values of either l_0 or FBR diameter. It reached its maximum value of about 1.0 when the value of $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ was around 80 h^{-1}. This relation had almost the same tendency as that obtained in the shaking flask system.[4] From these experimental results, it could be considered that $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ defined as Eq. (4) is a criterion of the production efficiency of the FBR system.

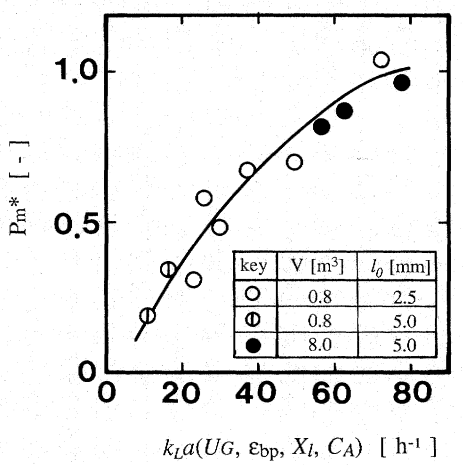

Fig. 6. Relation between relative penicillin yield, P_m^* and k_La (U_G, ε_{bp}, X_l, C_A)

The operational conditions which allowed $k_La(U_G, \varepsilon_{bp}, X_l, C_A)$ to be 80 h^{-1} were obtained in the region where ε_{bp} was 0.3 to 0.4 and U_G was 0.03 to 0.05 m·s^{-1}.

2.3 Semicontinuous penicillin production by repeated-batch operation

By varying the substrate concentration of the medium used in repeated-batch operation, the semicontinuous production of penicillin was studied. In RUN#1, the basal medium was used for repeated-batch operation, while in RUN#2 the concentration of lactose and that of corn steep liquor of the medium were reduced to a concentration of 30 to 50% of the basal medium.

The experimental results of RUN#1 and RUN#2 are shown in **Figs. 7** and **8** respectively. From Fig. 7, it is clear that the relative production yield of penicillin Y_m^* in each repeated-batch phase was maintained at almost the same value as that obtained in the initial batch culture. Here, Y_m^* is the maximum yield of

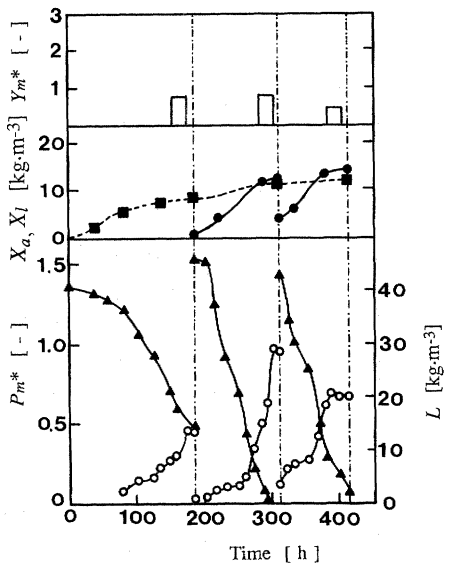

Fig. 7. Time course for repeated-batch production of penicillin in RUN#1
Y_m^*: relative amount of penicillin yield, P_m^* (○): relative amount of penicillin produced, L (▲): lactose concentration, X_C (■): adhering cell mass concentration, X_l (●): suspended cell mass concentration

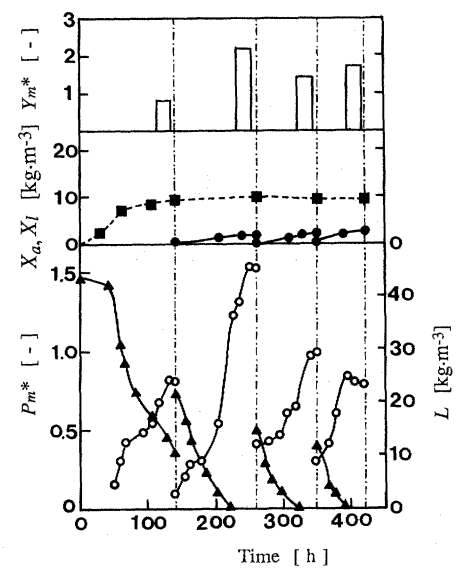

Fig. 8. Time course for repeated-batch production of penicillin in RUN#1
(These symbols are the same as shown in Fig. 7)

penicillin for lactose utilized, which was normalized by the value obtained in the control fermentation, as well as P_m^*. In this experimental condition, a relatively high amount of growth of leaked cells in the culture broth was observed during the repeated-batch phase. In RUN#2, X_l was maintained much lower than in RUN#1 and Y_m^* was about twice that in the initial batch culture. Furthermore, the cultivation time to attain maximum production of penicillin was shorter in each repeated-batch operation than that in the initial batch culture.

These results reveal that the activity of penicillin production by the adhered cells in the carriers, which grew in the initial batch operation, was maintained through the repeated-batch operation period. Comparison of the results of RUN#1 and RUN#2 led us to the hypothesis that the substrate concentration of the medium for repeated-batch culture regulated the metabolism of adhered cells in the carriers. That is, growth of cells was repressed and production of penicillin was accelerated under the condition of poor substrate concentration.

Conclusion

By using urethane foams as carriers for *Penicillium chrysogenum*, a novel cultivation method for effective repeated-batch production of penicillin in a fluidized-bed bioreactor (FBR) was developed.

The results are summarized as follows:

1) By studying the effect of the volumetric mass transfer coefficient, k_La, on the production level of penicillin in the FBR, it was found that production was closely related to k_La. It was found that a large amount of penicillin was produced in the operational region where ε_{bp} was 0.3 to 0.4 and U_G was 0.03 to 0.05 m·s^{-1}.

2) The adhered cells in the urethane foam carriers could be used repeatedly for semicontinuous production of penicillin without loss of activity. Furthermore, about twice the penicillin production yield for lactose utilized was obtained by using a medium in which substrate concentration was less than 50% of the basal medium for repeated-batch cultivation.

Nomenclature

C_A	=	concentration of antifoam reagent	[m^3·m^{-3}]
k_La	=	apparent volumetric mass transfer coefficient of oxygen	[h^{-1}]
L	=	concentration of lactose	[kg·m^{-3}]
l_O	=	side length of urethane foam cube	[m]
P	=	concentration of penicillin produced	[kg·m^{-3}]
P_m^*	=	relative maximum concentration of penicillin produced	[—]
U_G	=	superficial velocity of gas	[m·s^{-1}]
X_a	=	concentration of cell mass adhered in urethane foam	[kg·m^{-3}]
X_l	=	concentration of cell mass leaked from carriers	[kg·m^{-3}]
Y_m^*	=	relative production yield of penicillin for lactose utilized	[—]
α	=	coefficient defined by Eq. (1)	[—]
β	=	coefficient difined by Eq. (2)	[—]
γ	=	coefficient defined by Eq. (3)	[—]
ε_{bp}	=	volumetric ratio of carriers to working volume of FBR	[—]

Literature Cited

1) Aiba, S., A. E. Humphrey and N. F. Milis: "Biochemical

Engineering," p. 184, University of Tokyo Press, Tokyo (1973).
2) Deo, Y. M. and G. M. Gaucher: *Biotechnol. Bioeng.*, **26**, 285 (1984)
3) Endo, I., T. Nagamune and I. Inoue: *Kagaku Kōgaku*, **49**, 293 (1985)
4) Endo, I., T. Nagamune, N. Kato, M. Nishimura and T. Kobayashi: *Bioprocess Engineering*, **3**, 63 (1988).
5) Fan, L. T. and M. P. Newcomer: *Adv. Biotechnol.* (Proc. Int. Ferment. Sympo., 6th, 1980), **1**, 643 (1981).
6) Gbewonyo, K. and D. I. C. Wang: *Biotechnol. Bioeng.*, **25**, 2873 (1983).
7) Kim, J. H. and D. K. Oh: *Biotechnol. Bioeng.*, **28**, 1838 (1986).
8) König, B., K. Schügerl and C. Seewald: *Biotechnol. Bioeng.*, **24**, 259 (1982).
9) Nagamune, T., I. Endo, N. Kato, M. Nishimura and T. Kobayashi: *Bioprocess Engineering*, **3**, 173 (1988).
10) Van't Riet, K.: *Ind. Eng. Process Des. Dev.*, **18**, 357 (1979).