0273-2289/88/1902—0209$02.40

Microbial Conversion of 4-Oxoisophorone by *Thermomonospora curvata* Using an Air-Bubbling Hollow Fiber Reactor

KOJI SODE,[1] MINORU HONDA,[3] YOICHI MIKAMI,[3] TSUYOSHI YANAGIMOTO,[4] AND ISAO KARUBE[1,2,*]

[1]Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153; [2]Research Laboratory of Resources Utilization, Tokyo, Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, 227; [3]Central Research Institute, Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku, Yokohama, 227; and [4]Research Department, Engineering Division, NOK Corp., 4-3-1 Tsujido-shinmachi, Fujisawa, 251, Japan

ABSTRACT

Microbial conversion of 4-oxoisophorone (OIP) by thermophilic bacterium *Thermomonospora curvata* was attempted in a continuous process.

The correlation between cell growth and microbial conversion was first examined in a batch culture. The results indicated that this microbial conversion was strongly dependent upon cell growth. In a continuous microbial conversion of OIP using a continuous stirred tank reactor, the cell density in the reactor seemed to be the limiting factor in the OIP conversion. Therefore, we developed an air-bubbling hollow fiber reactor to achieve a high density culture. By using this bioreactor, more than 3.3 times higher productivity was achieved. In addition, during the process, only a slight cell contamination to the product was observed. Therefore, this bioreactor is suitable for the continuous microbial conversion, considering further downstream processes and high productivity.

*Author to whom all correspondence and reprint requests should be addressed.

Index Entries: Microbial conversion; thermophilic bacteria; hollow fiber reactor; *Thermomonospora curvata*; 4-oxoisophorone.

INTRODUCTION

Immobilization of microorganisms is widely accepted as a method having many advantages. Particularly, when keeping a high density of cells in a reactor, even at a high dilution rate to subsequently achieve a high productivity. Some microbial processes are known to be associating with cell growth. In such cases, if we apply the immobilization techniques for the processes, cell growing at the outside of the immobilizing gel beads will cause the contamination of the product. In addition, by using entrapment methods for microorganisms immobilization, cells immobilized inside of the gel beads will not contribute to the bioprocesses because of the large diffusion barrier.

The application of membrane technology to bioprocesses is increasing. The production of high density culture of microorganisms by using membrane reactors, like crossflow filtration systems, has been studying by several researchers (*1–4*). Utilizing membranes with appropriate molecular cutoff, microorganisms can be maintained in a reactor even at a high dilution rate, and, also, no diffusion barrier of entrapped cell exists.

The authors have been studying the microbial conversion of terpenoids (*5–8*). The application of thermophilic bacteria to organic compound synthesis has many advantages because of the high temperature employed (*9–12*). We previously reported on the continuous conversion of 2,6,6,-trimethyl-2-cyclohexene-1,4-dione (4-oxoisophorone, OIP) to (6R)-2,2,6,-trimethyl-1,4-cyclohexane-dione((3 R)-dihydro-4-oxoisophorone DOIP) by *Thermomonospora curvata* using several bioreactors (*13*). Utilizing hollow fiber type reactor, the contamination by cells in the product was reduced, however, the productivity was not satisfactory. This was because of low cell viability.

In this study, we demonstrate a novel bioreactor for microbial conversion using hollow fiber. First, we investigate the correlation of cell growth and microbial conversion of OIP, and also demonstrate a continuous microbial conversion utilizing conventional continuous stirred tank reactor. Then, we show a continuous process using an air-bubbling hollow fiber reactor.

MATERIALS AND METHODS

Chemicals

4-Oxoisophorone (OIP) and (3R)-dihydro-4-oxoisophorone (DOIP) were supplied by Japan Tobacco Inc. All other chemicals were of reagent grade.

Microorganisms and Cultivation

Thermomonospora curvata JTS321 provided by Japan Tobacco Inc. was used in this study. The culture conditions are as reported in our previous study (*13*).

Microbial Conversion of OIP

Batch Culture

T. curvata was inoculated in a 500 mL flask containing 100 mL of medium. After cell growth was observed, 300 mg of OIP was added in the medium, and cultivation was continued. Varifying the time of OIP addition, the effect of cell growth on the conversion rate was examined.

Continuous Conversion Using Continuous Stirred Tank Reactor (CSTR)

A 1000 mL of CSTR (Marubushi Bioeng. Co. Ltd., Chiyoda-ku, Tokyo, Japan) with 500 mL of working volume was used in this study. The operational condition is shown in each figure.

Continuous Conversion Using Air-Bubbling Hollow Fiber Reactor

The hollow fiber reactor was supplied by NOK Corp. (Fujisawa-shi, Kanagawa, Japan). The reactor was constructed from polyvinyldifluoride fibers (molecular cut off; 400,000, surface area; 326.7 cm^2). *T. curvata* was inoculated in the extra capillary space (ECS; an internal space between packed hollow fibers and the shell of the reactor, working volume; 9.2 mL). The medium with 3 mg ml^{-1} OIP was continuously fed at ECS, and the product was recovered through the hollow fiber.

Analytical Methods

The analytical methods are as described in our previous study (*13*).

RESULTS AND DISCUSSION

Microbial Conversion of OIP in a Batch Culture

Figure 1 shows the time course of microbial conversion of OIP in a batch culture. Thirteen h after the addition of OIP, the conversion extent was 85%. At this point, the byproduct, cis-4-hydrooxy-3,3,5-trimethylcyclohexanone was less than 5% of total OIP derivatives. The highest cell density was achieved in 4 h after OIP addition. As can be seen from this figure, OIP conversion was mostly employed at the rapid growth phase. Therefore, this microbial conversion seems to be a growth associated type.

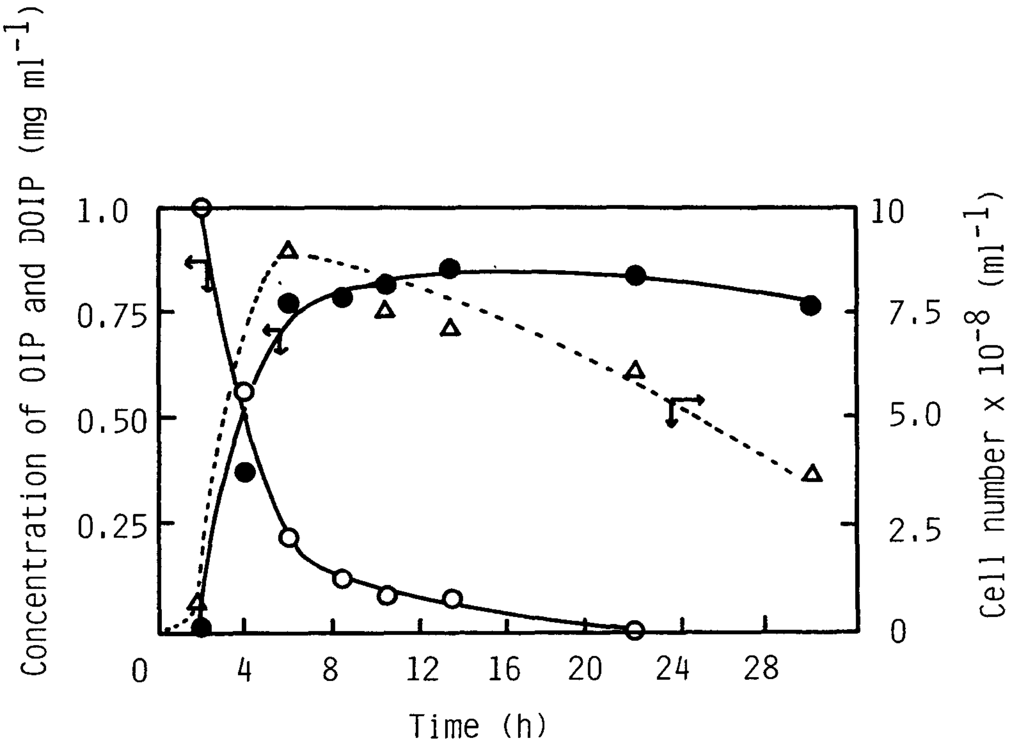

Fig. 1. Time course of OIP conversion. (–○–); OIP concentration, (–●–); DOIP concentration, (--△--); cell concentration.

OIP was added at several points in the growth phase of *T. curvata* batch culture, and the correlation between cell growth and microbial conversion was investigated. Figure 2 shows the time course of production rate of DOIP per cell. The maximum value was observed in the early phase of the batch culture.

Figure 3 represents the correlation between specific growth rate and DOIP production rate per cell. A good linear correlation was observed in this figure. Thus, this microbial conversion is strongly dependent on cell growth. This suggests that it is essential to keep a high cell growth rate to achieve a high production rate of DOIP in a continuous process.

Continuous Microbial Conversion of OIP Using CSTR

The continuous microbial conversion of OIP was then attempted using CSTR.

Figure 4 shows the effect of dilution rate on the cell number in a reactor. With the increase of dilution rate, cell number decreased. However, the dependency of cell number on dilution rate was not marked, as far as can be seen for the dilution rates we examined. It is apparent, that at the dilution rates we examined, no significant washout of cell has occurred.

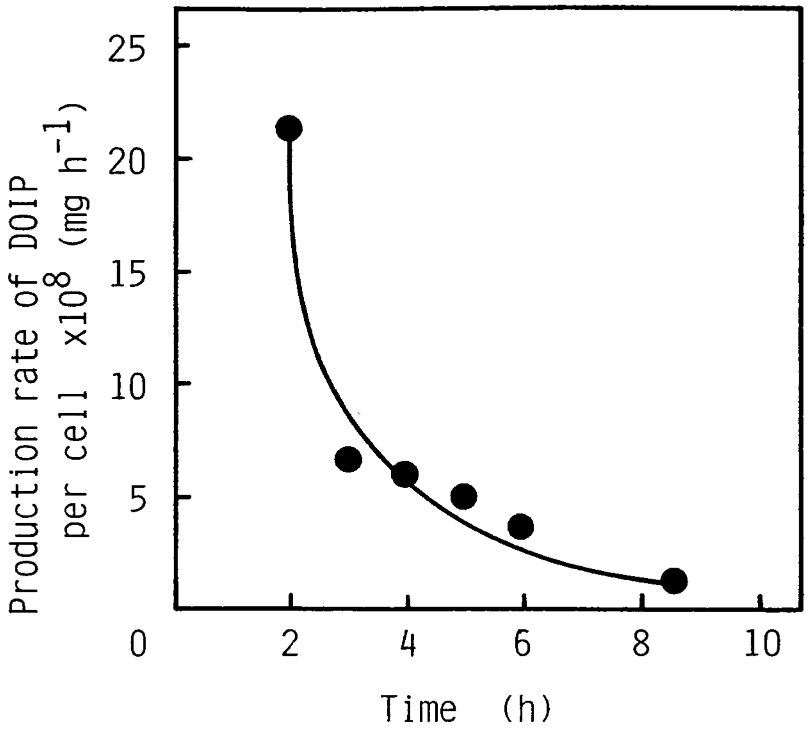

Fig. 2. Time course of production rate of DOIP per cell.

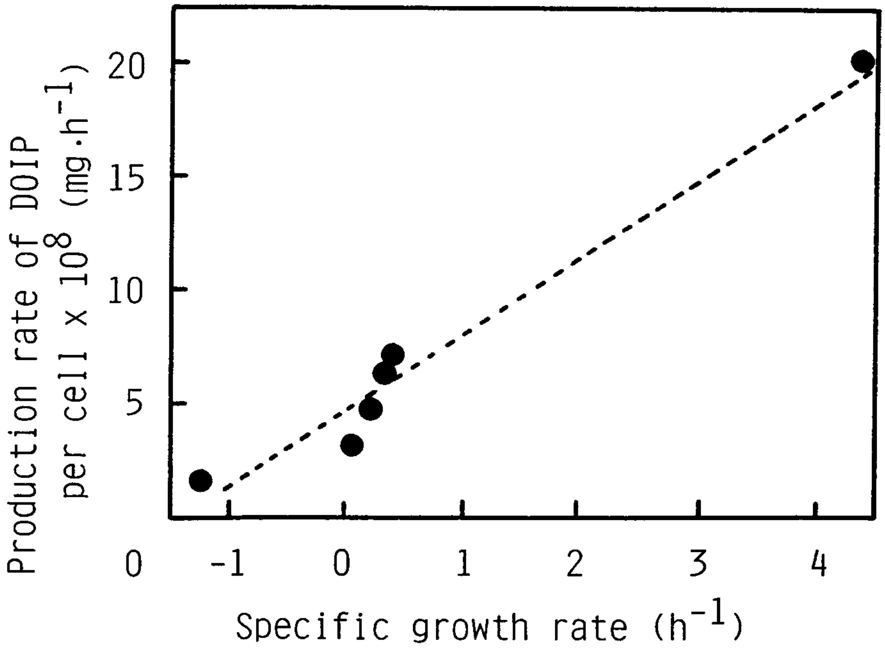

Fig. 3. Correlation between specific growth rate and DOIP production rate.

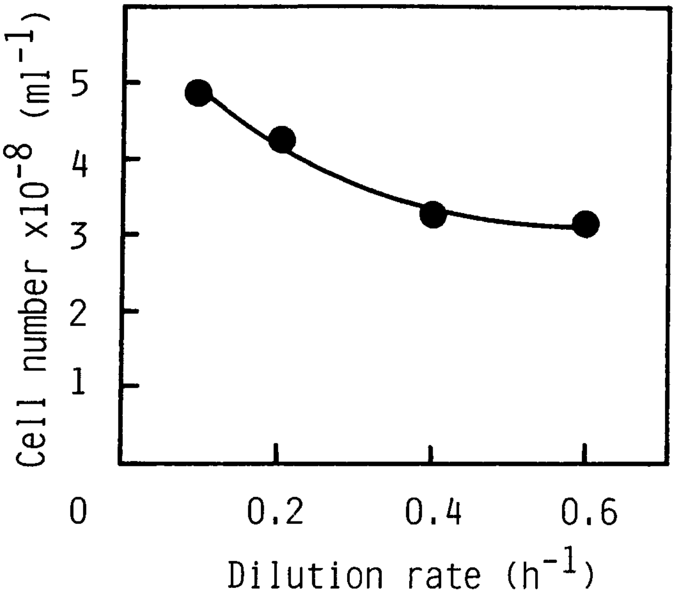

Fig. 4. Effect of dilution rate on cell concentration during OIP continuous conversion using CSTR. Temperature; 50°C, aeration; 500 mL min^{-1}, OIP concentration; 3 mg mL^{-1}.

The effect of dilution rate on OIP conversion extent is shown in Fig. 5. The maximum conversion extent was observed at dilution rate 0.1–0.2 h^{-1} (65%). At a higher dilution rate than 0.2 h^{-1}, the conversion extent decreased.

Figure 6 represents the effect of dilution rate on DOIP productivity. At a dilution rate 0.4 h^{-1}, the highest DOIP productivity was achieved (430 mg h^{-1} L^{-1}).

Therefore, the extent of conversion and productivity were strongly dependent on the dilution rate. If we intend the improvement of the productivity of DOIP, the following two approaches might be attempted. First is to increase the catalytic activity of cells and second is to increase the amount of the cells in the reactor.

If we attempt to increase the catalytic activity of cells, we have to increase the specific growth rate according to the results from Fig. 3. However, the specific growth rate in the reactor was dominated by the dilution rate. Considering the results from Fig. 6, the increase in the dilution rate higher than 0.6 h^{-1} will cause the decrease in the productivity of DOIP. It would be owing to the retention time of OIP in the reactor.

Considering the results from Fig. 4, no marked cell washout was observed. Thus, cell concentration in the reactor was in its maximum at the dilution rate we examined. This suggests that it would be difficult to achieve the significant increase in the cell concentration using this reactor.

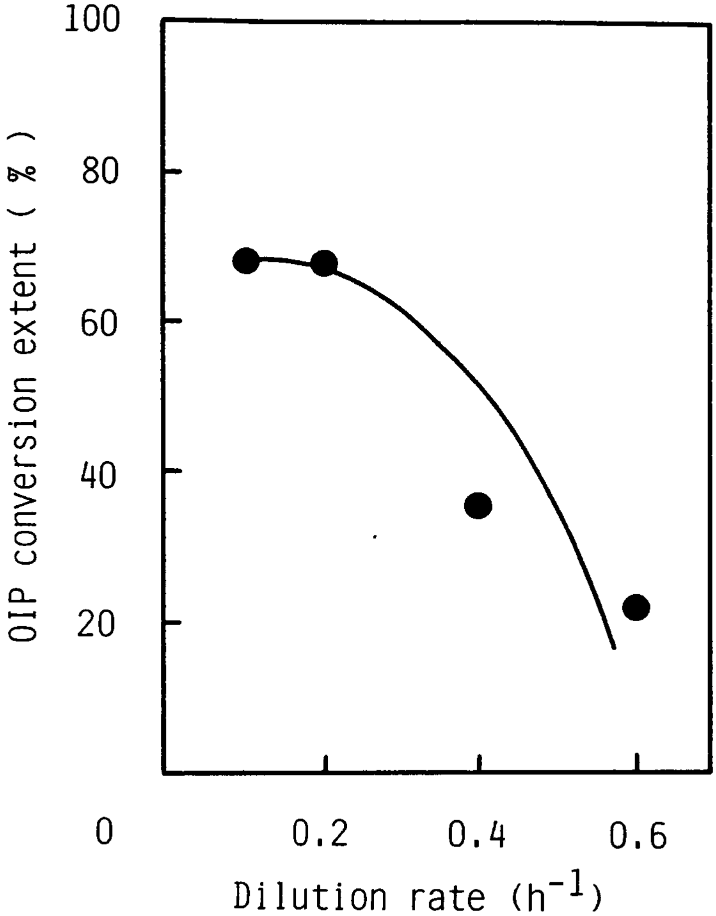

Fig. 5. Effect of dilution rate on OIP conversion extent. Operational condition is shown in Fig. 4.

Therefore, it might be impossible to improve the productivity of DOIP as far as using this reactor. In the other words, we have to develop a novel bioreactor system to achieve a higher productivity of DOIP.

Continuous Microbial Conversion of OIP Using an Air-Bubbling Hollow Fiber Reactor

To achieve a higher efficiency of OIP conversion, we then investigated an air-bubbling hollow fiber reactor. The schematic diagram of this reactor is shown in Fig. 7. The higher cell density than those achieved in a CSTR was expected, since the molecular cutoff of the membrane used in the hollow fiber was small enough to keep cells in a reactor. Therefore we attempted the higher dilution rate than we examined in a process using CSTR.

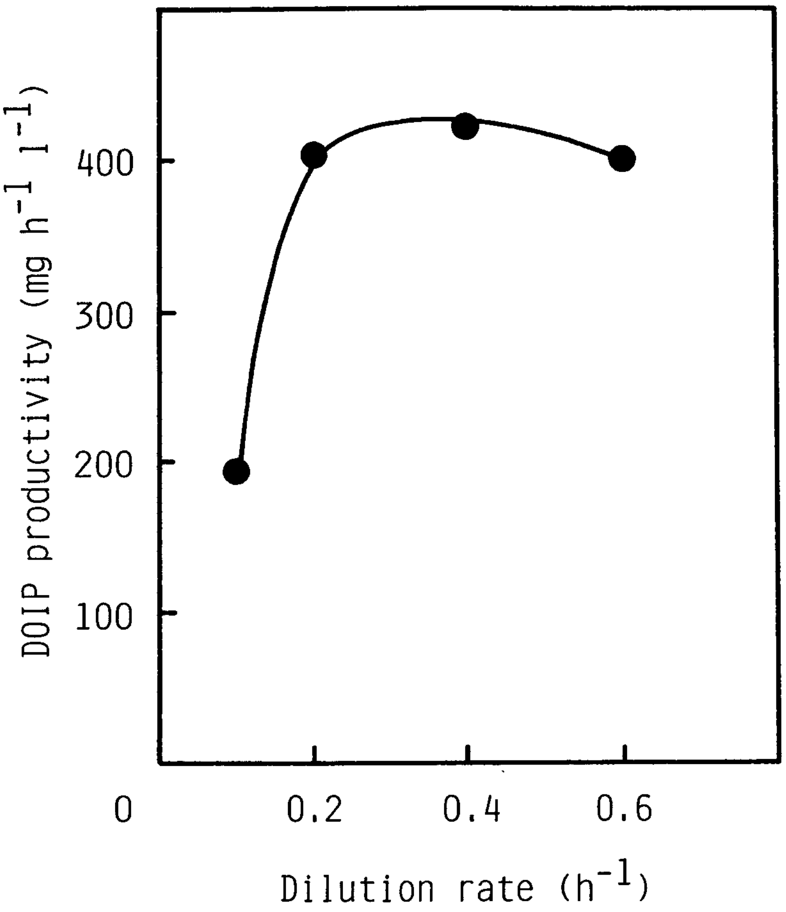

Fig. 6. Effect of Dilution rate on DOIP productivity. Operational condition is shown in Fig. 4. DOIP productivity is expressed as the amount of DOIP produced in 1 h/1 L working volume reactor.

The effect of dilution rate on OIP conversion extent is shown in Fig. 8. The highest conversion extent (70%) was observed at dilution rate 0.24 h^{-1}. With the increase of the dilution rate, the conversion extent decreased. Compared with the conversion extent dependency on dilution rate of a process using CSTR, however, it was significantly diminished by using hollow fiber reactor.

Figure 9 shows the effect of dilution rate on DOIP productivity. As far as the dilution rate we examined, no maximum productivity was observed. However, with the increase of dilution rate, the productivity also increased. The highest productivity (1400 mg h^{-1} L^{-1}) was achieved at dilution rate 2.5 h^{-1}. This productivity was approximately 3.3 times higher productivity than the maximum productivity achieved using CSTR. Theoretically, using CSTR, an increased dilution rate up to 4 h^{-1}

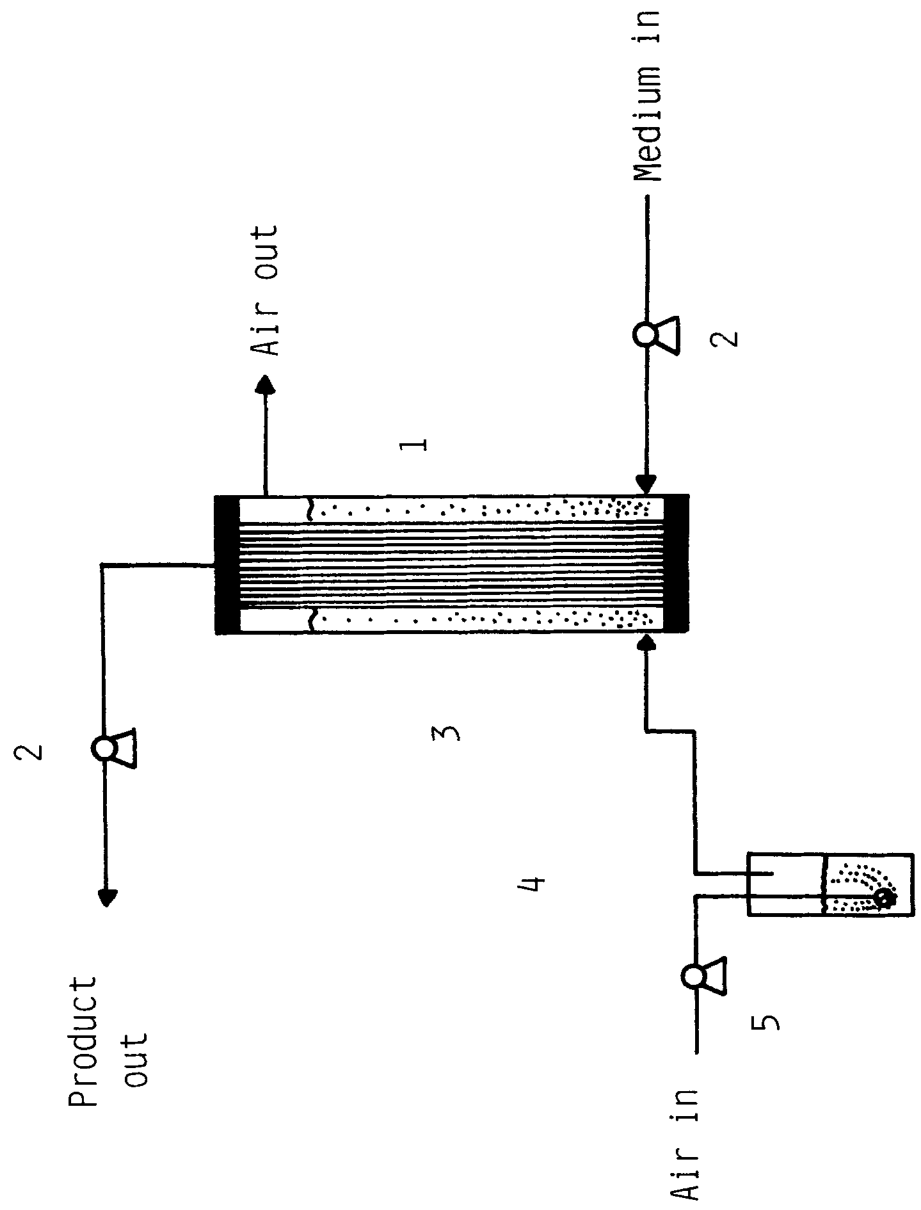

Fig. 7. Schematic diagram of an air-bubbling hollow fiber reactor. 1; hollow fiber, 2; peristaltic pump, 3; cells in ECS, 4; humidifier, 5; air pump.

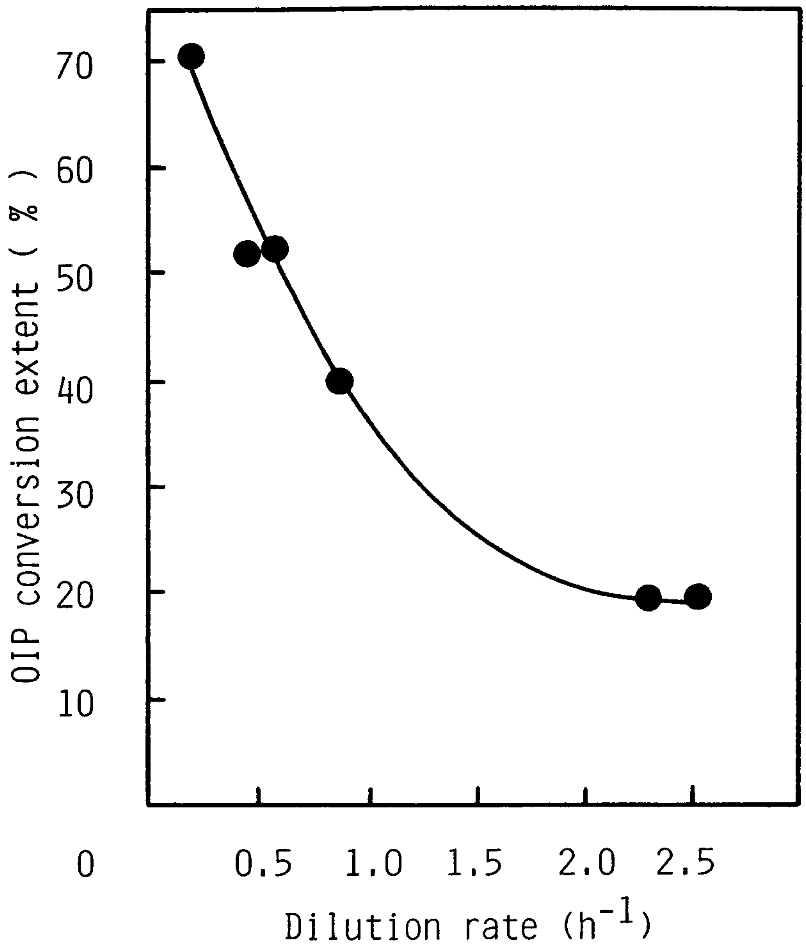

Fig. 8. Effect of dilution rate on OIP conversion extent (air-bubbling hollow fiber reactor) Temperature; 50°C, aeration; 25 mL min^{-1}, OIP concentration; 3 mg mL^{-1}.

could be employed (Fig. 3). However, as can be seen in Fig. 6, dilution rates higher than 0.6 h^{-1} will cause lower productivity. Since the product was recovered through the hollow fiber, no cell washout has occurred even at such a high dilution rate. Subsequently, a high productivity was achieved by using air-bubbling hollow fiber reactor.

In addition, the product without cell contamination is highly desirable, when considering further downstream processing. During continuous conversion, a small amount of cell leakage in the product was observed. This was because of the lack of mechanical sealing or some crack in the hollow fiber. However, it can be improved by further optimization of the materials. Therefore, these types of reactors using hollow fibers are suitable for continuous microbial conversion associating cell growth.

To improve productivity, one should optimize the microenvironment to maintain an ideal growth rate, since the cells' growth rate is con-

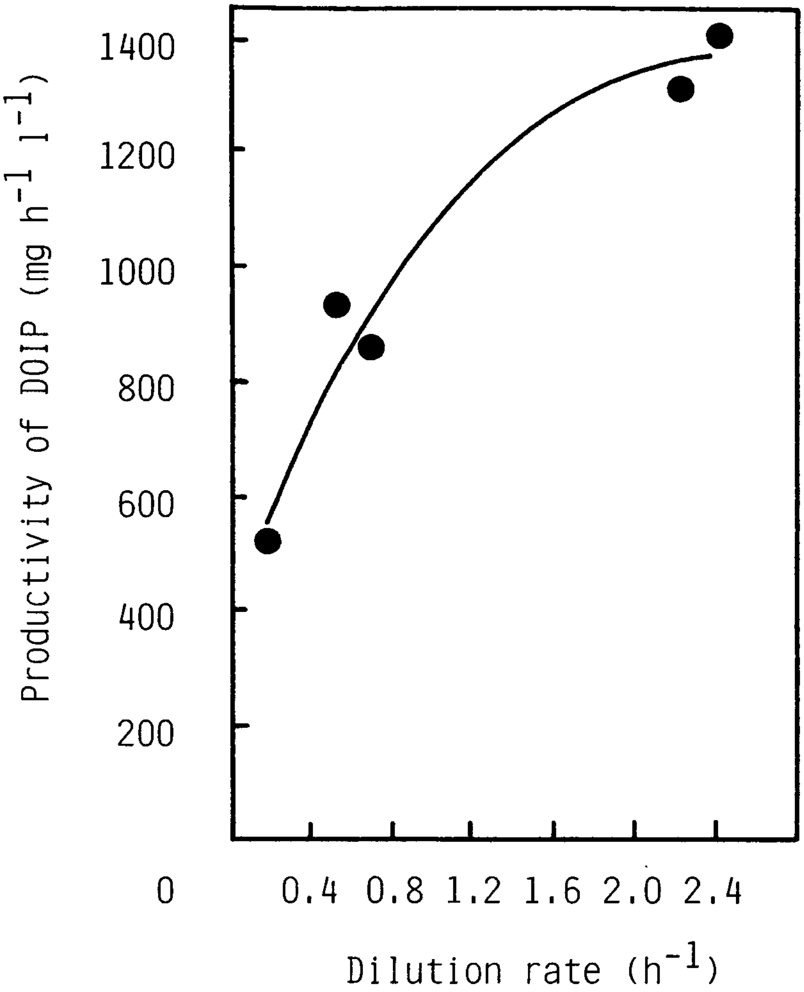

Fig. 9. Effect of dilution rate on DOIP productivity. Operational condition is shown in Fig. 8. DOIP productivity is expressed as the amount of DOIP produced in 1 h/1 L working volume reactor.

trolled only by the changing dilution rate. However, the correlation between dilution rate and specific growth rate was applied for the continuous process where the mass balance is controlled by the dilution rate at the steady state. In the air-bubbling hollow fiber reactor, cells were maintained in the reactor throughout the process and never washed out. As far as judging from the results obtained in this study, the high density culture system caused a high efficiency of microbial conversion. Therefore, the microenvironment seemed to be suitable for cell growth.

By controlling and optimizing pH, cell density, and dissolved oxygen in the extracapillary space of the reactor, higher productivity can be expected.

ACKNOWLEDGMENT

We thank Vincent M. McNeil for help in compiling in this paper.

REFERENCES

1. Holst, O., Hansson, L., Berg, A. C., and Matiasson, B. (1985), *Appl. Microbiol. Biotechnol.* **23,** 10.
2. Taniguchi, M., Kotani, N., and Kobayashi, T. (1987), *J. Ferment. Technol.* **65,** 179.
3. Taniguchi, M., Kotani, M., and Kobayashi, T. (1987), *Appl. Microbiol. Biotechnol.* **25,** 434.
4. Iijima, S., Kawai, S., Mizutani, S., Taniguchi, M., and Kobayashi, T. (1987). *Appl. Microbiol. Biotechnol.* **26,** 542.
5. Mikami, Y., Watanabe, E., Fukunaga, Y., and Kisaki, T. (1978), *Agric. Biol. Chem.* **42,** 1075.
6. Mikami, Y., Fukunaga, Y., Arita, M., and Kisaki, T. (1981), *Appl. Environ. Microbiol.* **41,** 610.
7. Mikami, Y., Fukunaga, Y., Hieda, T., Obi, Y., and Kisaki, T. (1981), *Agric. Biol. Chem.* **45,** 331.
8. Mikami, Y., Fukunaga, Y., Arita, M., Obi, Y., and Kisaki, T. (1981), *Agric. Biol. Chem.* **45,** 791.
9. Hori, N., Hieda, T., and Mikami, Y. (1984), *Agric. Biol. Chem.* **48,** 123.
10. Sonnleitner, B. and Fiechter, A. (1983), *Trends in Biotechnol.* **1,** 74.
11. Sonnleitner, B., Giovanni, F., and Fiechter, A. (1985), *J. Biotechnol.* **3,** 33.
12. Sonnleitner, B. and Fiechter, A. (1986), *Appl. Microbiol. Biotechnol.* **23,** 424.
13. Sode, K., Kajiwara, K., Tamiya, E., Karube, I., Mikami, Y., Hori, N., and Yanagimoto, T. (1987), *Biocatalysis* **1,** 77.