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Bioprocesses and Applied Enzymology

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Editorial

This volume of Advances in Biochemical Engineering/Biotechnology as well as the following one are dedicated to Professor Armin Fiechter on the occasion of his 65th birthday. Contributions were solicited from Armin's colleagues but were limited to subjects which related to the biotechnology scope of this series.

One might judge a successful scientist by several criteria and Armin fullfills them all. One criterion of success is one's record of scientific achievement. Armin's work on yeast physiology, process development and instrumentation has been very fruitful and the necessity of adopting new technology or applying concepts from other disciplines has never hindered progress in his laboratory. One very important measure of achievement and the one which is likely to be the most important in both professional and human terms, is the training of successful students. Armin is one of the most eminent promoters of biotechnology in Switzerland and abroad and he was instrumental in establishing the Institute for Biotechnology af the ETH in 1982 of which he is still director. By pioneering the combination of fundamental and applied aspects he has greatly influenced the advancement of biotechnology in teaching and research in Switzerland.

At the age of 65, the pace of Armin's activities shows no sign of slackening. At the latest count there are some 20 doctoral students working under his supervision. He is currently the editor-in-chief of the Journal of Biotechnology and of this series, both of which were co-founded by him and have gained momentum under his influence.

This special issue is dedicated to Armin Fiechter with admiration.

Zürich, July 1990 Jakob Reiser

Armin Fiechter

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A Tubular Bioreactor for High Density Cultivation of Microorganisms

Isao Endo[1], Teruyuki Nagamune[1], Susumu Tachikawa[2], Hideki Inaba[2]

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By simulating the functions of the animal intestine, the authors have developed a novel tubular bioreactor (TBR) which is capable of containing both the reaction and separation of products in a single system. This reactor consisted of inorganic ultra filtration membrane modules in the primary part of the system, a heat exchanger and a recycling pump.

The operation characteristics of the TBR were studied by cultivating *Lactobacillus casei* at a laboratory scale. The cell density obtained was up to 10 times higher than the density obtained by using the conventional jar fermentor. Furthermore, 40 g l^{-1} of cell mass was obtained in only 14 hours with 20 l of fresh medium when the dilution rate was increased according to the cellular growth. Afterwards, the cultivation time and the volume of fresh medium were reduced to 44% and 74%, respectively, of the values in the cultivation operation at constant dilution rate.

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1 Introduction

First of all, we wish to express our heartfelt congratulations to Professor Armin Fiechter on the occasion of his 65th birthday. Recognizing the importance of bioreactors in the field of bioprocesses, Professor Fiechter has for many years been at the forefront in developing bioreactors. The outcome of this research is condensed in his recent review paper of "Physical and Chemical Parameters of Microbial Growth" [1].

The separation-capable bioreactor being currently developed integrates both the reaction process and separation in a single system and has attracted the attention of many biochemical engineers around the world [2, 3]. Due to the characteristics of the reactor, high density cultivation of the cells is possible overcoming the product inhibition which is inherent in the microbial growth reaction. The following three categories of reactors are currently being developed:

1) A bioreactor which is composed of a jar fermentor and separation unit [4], such as a centrifuge and a settler.
2) A bioreactor in which the biocatalysts are immobilized in its primary part [5].
3) A bioreactor which encloses and circulates the biocatalysts inside a membrane modules [2, 6, 7].

The former two types of reactors have been developed so far. However the last type of reactor has never been achieved because of difficulties with the membrane modules and their effective operation. The authors have succeeded with this type of reactor by producing a TBR [8]. By incorporating, as the primary part of the reactor, an inorganic UF membrane which has advantages in sterilizability, in chemical properties and in filtration performance, we have made the TBR.

The aim of this paper is to introduce the design and operational characteristics of this TBR and how it is applied to the high density cultivation of *Lactobacillus casei*.

2 Design of the TBR

2.1 Membrane Module

There are various membrane materials which may be used for the TBR. The requirements for the membrane materials are as follows:

(1) Durability against steam sterilization; i.e. bioreactor elements are needed which are durable against repeated steam sterilization.
(2) Full recovery of filtration flux by cleaning. The decrease of a filtration flux caused by fouling with chemical substances present in the culture medium or with microorganisms affects the efficiency of the membrane bioreactor. This situation requires that the membrane flux should be recovered fully by cleaning.

(3) Sanitary safety. The membrane module must be designed properly and must be made of appropriate materials so that any contamination from it is avoided completely [9].

The current advances in membrane manufacturing technology are remarkable, though organic membranes still seem to have some problems concerning the above mentioned requirements [9]. On the other hand, inorganic membranes which satisfy these requirements have been developed. Taking these requirements into consideration, the authors have used an inorganic membrane module made of zirconium oxide for the design of the separation-capable bioreactor.

2.2 Constitution and Characteristics of the TBR

As indicated in Fig. 1 [3], a conventional membrane reactor consists of a jar fermentor and a membrane module like a hollow fiber type or a spiral type made of organic membranes. Generally speaking, the volume of the inorganic membrane module per unit area of filtration surface is larger than the one of the organic membrane module. Therefore, when this module is used as the separation

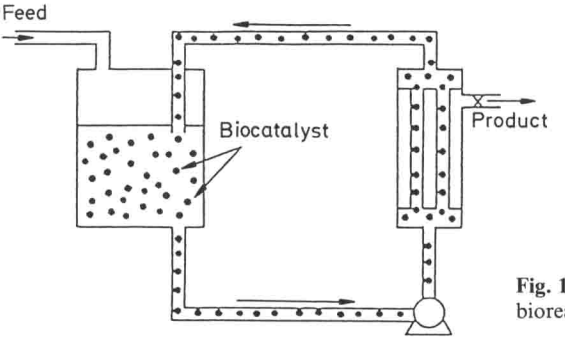

Fig. 1. Model of a typical membrane bioreactor

unit, the working volume of the separator becomes significantly larger. Since the ratio of the fermentor volume to the separator volume decreases drastically proportionally to the increase of filtration surface area per unit volume of the fermentor, the fermentor loses its role as the site of reaction. Taking into consideration the characteristics of the inorganic membrane, we have developed a closed-loop tubular bioreactor which consists solely of an inorganic membrane module, a heat exchanger, a recirculating pump and pipes that connect the above components. The concept of the TBR is illustrated in Fig. 2. We can combine the TBR with a degassing device and an oxygen supply for aerobic cultivation use.

Since the TBR is a closed-loop tubular reactor, it has the following characteristics:

(1) The TBR is easy to scale up due to its simple configuration.

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(2) As the TBR is operated under a pressure of 2 to 4 kg cm^{-2} for cross flow filtration, contamination can easily be avoided and the oxygen concentration and its effective use are high.

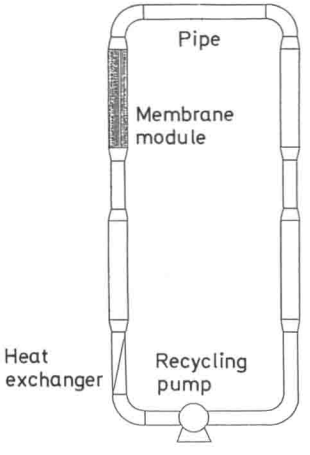

Fig. 2. Concept of a Tubular Bioreactor

3 Experimental Details

3.1 Equipment

In order to test the basic performances of the TBR, we set it up on a laboratory scale and applied it to the high density cultivation of *Lactobacillus casei*.

Figure 3 shows a flow diagram of the equipment. The total length of the closed-loop tube was 12 m and the reaction volume was 2.6 l. We used four UF membrane modules in which a tube with an internal diameter of 6 mm, an external diameter of 10 mm and a length of 1070 mm was introduced.

During circulation, the culture broth was filtered through the membrane tangentially and the product, lactic acid here, was removed. The rate of filtration was controlled automatically by a computer which was connected with a load cell.

3.2 Methods

3.2.1 Test Organism and Composition of the Basal Culture Medium:

Lactobacillus casei was used as a test organism in these experiments. The composition of the basal culture medium is shown in Table 1.

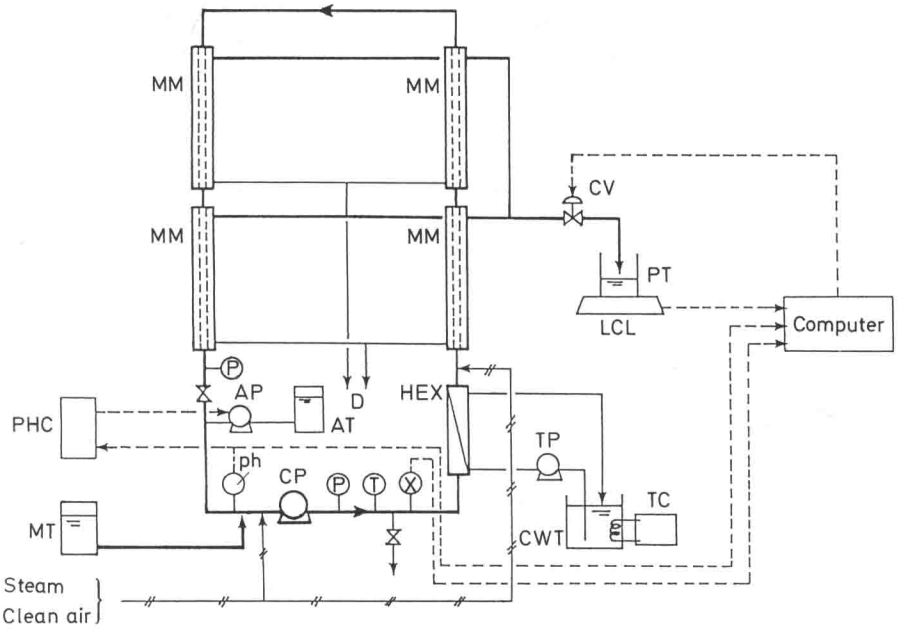

Fig. 3. Flow diagram of the TBR system; MM: membrane module CP: circulating pump HEX: heat exchanger, P: pressure gage, ph: pH sensor, T: thermometer, X: turbido sensor, pHC: pH controller, CV: control valve, LCL: load cell, TC: thermostat, AT: reservoir of alkali-sol., MT: reservoir of fresh medium, PT: reservoir of filtrate, AP: pump for alkali supplying, D: drain

Table 1. Composition of the basal medium

Substrate	Amount
Glucose	25 or 40 g l^{-1}
C S L*	100 or 160 g l^{-1}
K_2HPO_4	1 g l^{-1}
KH_2PO_4	1 g l^{-1}
$MnSO_4$	0.06 g l^{-1}

* Corn steep liquor

3.2.2 Experimental Conditions

3.2.2.1 Batch Culture

The TBR is usually operated under conditions appropriate for cross flow filtration, i.e. the flow rate on the surface of the membrane is 2 to 4 m s^{-1} and the average pressure operated is 2 to 4 bar. We studied the influences of operating conditions, such as the reactor characteristics derived from the

closed-loop tubular type, the shear stress by the pump and other operating variables, on the microbial reaction. In order to elucidate the influence of the operational conditions on the microbial reaction, we conducted batch culture experiments in the TBR and compared the results with the ones in a mini jar fermentor (2 l working volume). The culture temperature, the initial pH value and the initial concentration of glucose in the medium were kept at 35 °C, 6.8 and 22 g l^{-1}, respectively. The pH was not controlled during batch culture.

3.2.2.2 Continuous Culture with Cross-Flow Filtration

The temperature and pH were kept at 35 °C ± 0.5 °C and 6.5 ± 0.2, respectively. Batch cultivation was carried out before continuous operation in order to increase the cell concentration. The batch culture was started at the initial glucose concentration of 40 g l^{-1}; when the glucose had been consumed, fresh medium was fed continuously and cross-flow filtration was started. The glucose concentration of the feed medium was 25 g l^{-1}. We conducted the following two feeding operations. In the first method, the feed rate of the fresh medium was kept at a constant dilution rate, 0.32 h^{-1}. In the second, it was raised according to the growth of the cells.

3.2.3 Measuring Methods

The culture broth was sampled manually every four hours in order to measure the dry weight of the cell mass, the glucose concentration and the lactic acid concentration. The concentrations of glucose and that of lactic acid were measured by using the new glucostat reagent and the F kit lactic acid reagent, respectively. In addition, the optical density (OD) was measured by an on-line turbido sensor (Laserturbidimeter, Model LT201, ASR in Japan).

4 Results and Discussion

4.1 Comparison of Batch Culture Experiments Obtained with the TBR with Those of the Jar Fermentor

Figure 4 shows the comparison of batch culture experiments obtained in the TBR with those of a jar fermentor. Table 2 represents the specific rate of cellular growth, that of substrate consumption, and that of lactic acid production during the exponential growth phase of the cells, which were calculated by data shown in Fig. 4. As indicated in Fig. 4 and Table 2, no significant difference in the results between two cultivation systems were observed. This means that the operating conditions and configuration of the TBR did not affect the reactions of the lactic acid bacteria.

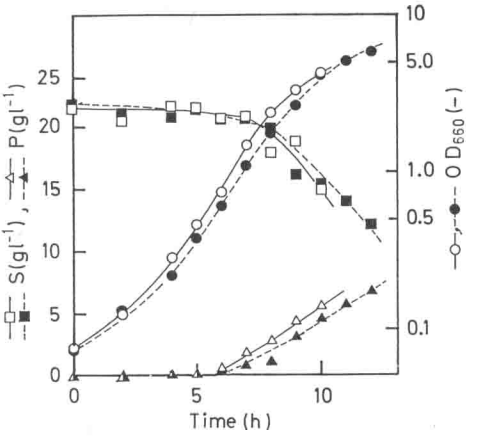

Fig. 4. Comparison of batch culture experiments obtained in the TBR with those obtained in a jar fermentor (JF)

Table 2. Values of the specific rates during the exponential growth phase of the cells in batch culture experiments

Reactor	μ (h^{-1})	ν (h^{-1})	π (h^{-1})
TBR	0.533	2.25	1.96
JF*	0.462	2.20	1.74

* Jar fermentor

4.2 Cultivation of *L. casei* with Cross-Flow Filtration at a Constant Dilution Rate

The feed rate of fresh medium F, was matched with the rate of filtration in order to maintain the working volume, V, constant. As the mixing inside the TBR is perfect, the material balance concerning glucose, cell mass and lactic acid are given by equations (1) to (3).

$$dS/dt = -\nu X + F/V \cdot (S_f - S) = -\nu X + D \cdot (S_f - S) \quad (1)$$

$$dX/dt = \mu X \quad (2)$$

$$dP/dt = \pi X - D \cdot P \quad (3)$$

where S_f is the glucose concentration in the feed of fresh medium, S is the glucose concentration in the reactor, X is cell mass concentration, P is the lactic acid concentration, D is the dilution rate, ν is the specific glucose consumption rate, μ is the specific growth rate and π is the specific lactic acid production rate. Specific rates ν, μ and π can be derived and calculated from the above equations.

$$\nu = -(dS/dt)/X + D \cdot (S_f - S)/X \quad (4)$$

$$\mu = (dX/dt)/X \quad (5)$$

$$\pi = (dP/dt)/X + DP/X\,. \quad (6)$$

Figure 5 shows the time course of S, X, P, feed rate of fresh medium, F, and amount of fresh medium supplied, $\int F dt$, under conditions where S_f is 25 g l^{-1} and D is 0.32 h^{-1}. The calculated results concerning ν, μ and π are shown in in Fig. 6.

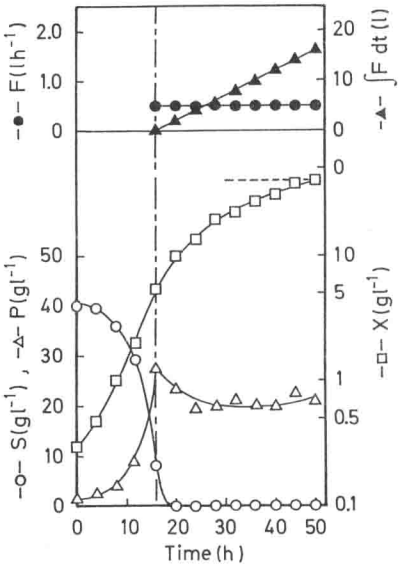

Fig. 5. Experimental results obtained by keeping the dilution rate constant

This figure shows that when S is decreased to nearly zero, the specific rates do not drop to zero immediately, but decrease gradually according to the lapse of time. When S and dS/dt in Eq. (4) are approximately zero, the specific glucose consumption rate, ν, is obtained by the following equation;

$$\nu = D \cdot S_f/X\,. \quad (7)$$

Equation (7) indicates that the glucose consumption rate, ν, is governed by the cell mass concentrations when $D \cdot S_f$ is constant. Since the specific growth rate, μ is influenced directly by the glucose consumption rate, μ is obviously also controlled by the cell mass concentration. This is the reason why the growth rate of the cell decreases when the cell mass concentration increases as shown in Fig. 5. From this consideration, it is suggested that, by increasing dilution rate according to the cellular growth, a high growth rate of the cell is to be expected.

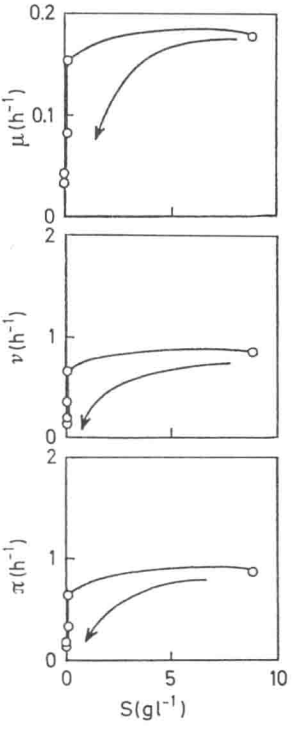

Fig. 6. Characteristic curves concerning to the specific growth rate of cells, μ, the rate of glucose consumption, ν, and the rate of lactic acid production, π *vs* the residual glucose concentration in the culture broth, S. The values were obtained under continuous cultivation conditions where the dilution rate was constant

4.3 Cultivation of *L. casei* with Cross-Flow Filtration by Increasing the Dilution Rate

On the basis of our knowledge mentioned above, we studied high efficiency and high cell density cultivation of *L. casei* with cross-flow filtration by increasing the dilution rate. From the stand point of efficient utilization of the feeding substrate, it is preferable that the glucose concentration in the reactor, S, should be kept at nearly zero. Under these conditions, the specific growth rate, μ, should be kept as high as possible to get a high cell mass concentration in a short time. Figure 6 shows that these requirements can be accomplished under the conditions that μ and ν are equal to 0.16 h^{-1} and 0.8 h^{-1}, respectively. Substituting this ν value and the value of S_f, 25 g l^{-1}, into Eq. (7), the dilution rate of fresh medium, D, is obtained by Eq. (8).

$$D = \nu \cdot X/S_f = 0.032X\,. \tag{8}$$

The cultivation of *L. casei* in the TBR was carried out controlling the dilution rate of fresh medium in proportion to the cell mass concentration. The cell mass concentration was estimated via a signal from the on-line turbido sensor. The experimental results obtained are shown in Fig. 7. The experiments

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