EQUIPMENT AND OPERATION FOR FERMENTATIVE PHB PRODUCTION USING GASEOUS SUBSTRATE TO GUARANTEE SAFETY FROM EXPLOSION

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Introduction

The hydrogen-oxidizing bacterium *Alcaligenes eutrophus* accumulates poly-β-hydroxybutyric acid (PHB), which is a raw material of biodegradable plastic in the cell, from H_2, O_2 and CO_2. We have already proved that PHB can be produced with high gas utilization efficiency in autotrophic cultivation of *A. eutrophus* using a recycled gas closed-circuit culture system[6, 7]. However, in this system, O_2 concentration of the used substrate gas mixture was 10 to 15%, which is within the range of gas detonation. To eliminate the danger of detonation, O_2 concentration in the system must be kept below the lower limit of explosion without reducing the rate of gaseous mass transfer into the medium. However, such condition could not be attained by using a conventional system. We newly built a bench-plant scale fermentation system with several safety devices, control systems and a specially designed agitation system, and performed the production of PHB from H_2, O_2 and CO_2 under safe culture condition by maintaining O_2 concentration below the lower limit of explosion.

1. Experimental

1.1 Microorganism and medium

The bacterial strain used was *Alcaligenes eutrophus* ATCC 17697^T and the medium was the same as previously reported[6].

1.2 Cultivation system

A schematic flow diagram of the bench-plant scale fermentation system with 2-dm^3 jar fermentor is shown in **Fig. 1**. The gas mixture (H_2:CO_2 = 9:1) stored beforehand in an iron-bell gas chamber (total volume: 450 dm^3) standing in saline solution was fed into the jar fermentor through a sterile filter (BLS Filter, Pall Co., Ltd., Japan) by a circulating pump (BA-106 type, Iwaki Co., Ltd., Japan), then returned to the chamber. Any change in pressure within the chamber due to gas consumption was compensated by rise of the saline water. This system is a type of recycled-gas closed circuit but different from the previous one on several points. Safety devices, gas control system and special agitation system were newly incorporated into the system, and pure O_2 gas was directly fed into the medium independently from the mixed gas. An internal pressure sensor connected to the gas releasing system and a combustible gas detector interfaced with a shut-down device were installed in the gas feed line and outdoors, respectively. All electrical equipment were explosion-proofed. The gas chamber and all associated equipment were installed outdoors and the pipes were treated with oil insulation.

1.3 Feeding of oxygen

The lower limit of detonation for O_2 in the gas mixture of H_2, O_2 and CO_2 was reported to be about 6%[12]. However, according to the result of our experiment using a modified Hempel's apparatus for gas determination (JIS K2301-1980), the gas mixture did not explode below 6.9% O_2. Thus, the danger of gas detonation is removed by maintaining the O_2 concentration in the system below 6.9%. Pure O_2 gas was directly fed into the fermentor through a solenoid-operated valve intermittently regulated by a DO_2 controller (DOC-2201, Biott Co., Ltd., Japan). The O_2 feed line was separate from that for the mixed gas.

1.4 Agitation system

As the O_2 concentration in the system was very low compared to that of other usual aerobic fermentations, the O_2 transfer rate (OTR) into the medium was so small that the productivities of biomass and PHB would also be very small. Thus, to obtain high OTR, a specially designed agitation system (EG-STAR, Biott Co., Ltd., Japan)[9, 10] was used. The volumetric transfer coefficient (K_La) of the fermentor with the agitation system measured by the sulfite oxidation method was very high (2970h^{-1} at 700 rpm, 30°C), and several times higher than that of a turbine impeller with six plane blades (data not shown). However, the use of this agitation system caused serious foaming of the broth, which resulted in broth loss via the fermentor gas outlet and cessation of

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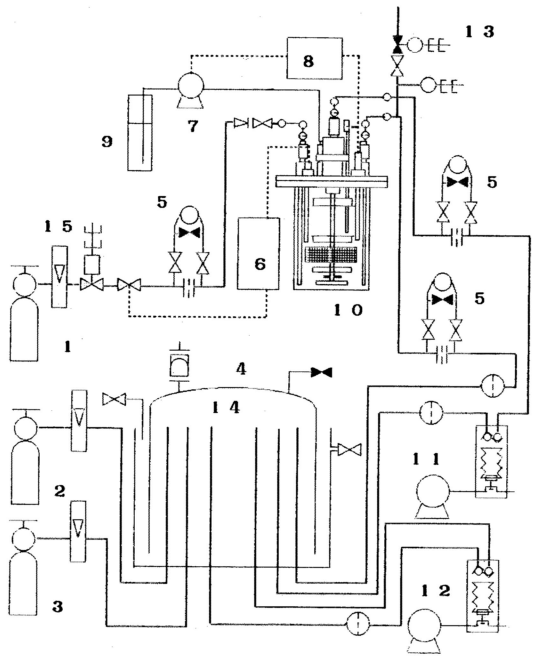


Fig. 1 Schematic flow diagram of the bench-plant scale recycled-gas closed-circuit culture system
1, O_2 cylinder; 2, H_2 cylinder; 3, CO_2 cylinder 4, gas chamber; 5, differential-pressure flowmeter; 6, DO_2 controller; 7, alkali feeding pump; 8, pH controller; 9, NH_4OH soln.; 10, fermentor; 11, circulating pump; 12, mixing pump; 13, internal pressure sensor; 14, saline replacement; 15, flowmeter

gas feeding because of blockage in the return line. Therefore, antifoaming reagent should be used to prevent the broth from foaming, although *A. eutrophus* was very sensitive to some usual antifoaming regents such as silicon oil, and addition of reagents often ended the fermentation. The antifoam reagent, A-nol (Biott Co.,Ltd., Tokyo) did not inhibit cell growth, and its use enabled high-density cultivation of the microorganism.

1.5 Culture condition and analysis

The preculture and analyses were carried out according to our previous reports[6, 7]. The main culture was carried out under following conditions: the working volume; 1 dm^3, agitation speed: 700 rpm, gas feeding rate: 2 dm^3min^{-1}, the pH: 7.0, temperature: 30°C. The composition of gas mixture in the return and feeding line, and in the chamber, was determined by gas chromatography.

2. Results and Discussion

The time course of a batch cultivation of *A. eutrophus* using the bench-plant scale fermentation system is shown in **Fig. 2**. The set point of the DO_2 controller was 1.8 ppm (equivalent to 5% in gas phase and the most suitable concentration for the cell growth[6] at the start of cultivation, and was manually lowered as the O_2 concentration in the return line increased. After biomass concentration increased to about 25 gdm^3, complete O_2 limitation was achieved by lowering the set point of DO_2

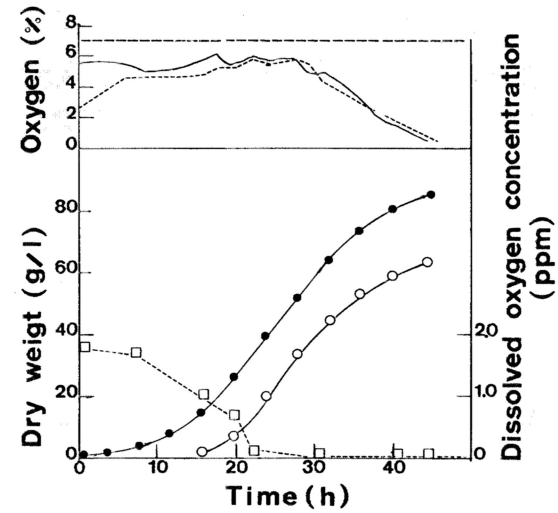


Fig. 2 Fermentation time course for autotrophic batch cultivation of *A. eutrophus* using the bench-plant scale fermentation system
(-----), oxygen concentration in feed line
(——), oxygen concentration in return line
(– – –), the lower limit of explosion
(●), biomass concentration
(○),PHB concentration
(□), dissolved oxygen concentration

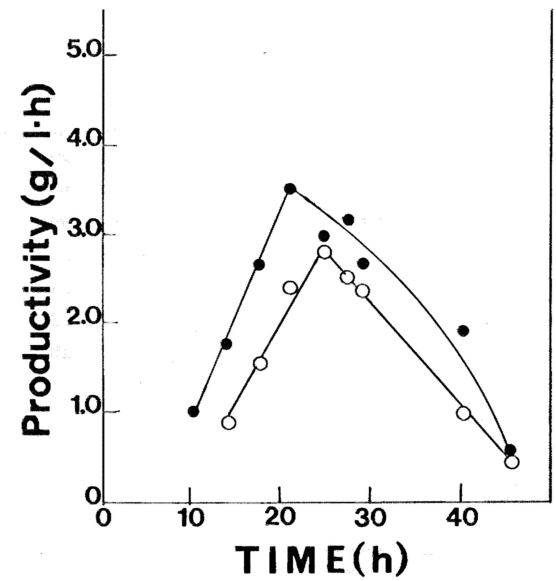


Fig. 3 Productivities of biomass and PHB in autotrophic cultivation of *A. eutrophus* using the bench-plant scale fermentation system
(●), biomass; (○), PHB

to 0.2 ppm, and PHB accumulation was promoted. After 45 hours of cultivation the fermentation ceased and the concentrations of biomass and PHB reached 85.7 gdm^{-3} and 61.5 gdm^{-3} respectively while the O_2 concentration was maintained below 6.9%. These final concentrations of biomass and PHB were much higher than those of previous studies for autotrophic batch culture of hydrogen-oxidizing bacteria[1, 3-5, 8, 11]. It was obvious that the very high K_La of the agitation system used allowed high product concentrations with relatively short fermentation time even under such a very low O_2

condition. **Figure 3** shows the productivities of biomass and PHB during the batch cultivation. Maximum productivity of PHB was 2.84 $gdm^{-3}h^{-1}$ at 24 hours cultivation, and that of biomass was 3.83 $gdm^{-3}h^{-1}$ at 20 hours. In this system, pure O_2 gas was fed to control the dissolved O_2 concentration at the set value of DO_2, 0.2 ppm in PHB accumulation phase. However, it was very difficult to balance the feeding rate of O_2 with the consumption rate by the cells, and O_2 concentration in the gas phase increased and approached the explosion limit. Hence, the feeding of O_2 was stopped after 30 h of cultivation.

The result of this experiment proved that PHB could be produced safely from H_2, O_2 and CO_2 at a high productivity by maintaining the O_2 concentration at a lower level than the explosion limit and using a reactor with very high K_La. For practical application of the system, the size of the gas chamber should be scaled down and the composition of the gas phase and the DO_2 concentration should be strictly controlled. Such control can be achieved by incorporating an on-line measuring system coupled with a computer control system.

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