# Production of α-Amylase by Immobilized Cells of *Aspergillus oryzae* in a Bubble Column Bioreactor

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α-Amylase production by *Aspergillus oryzae* IFO 30113 immobilized on a macroporous cellulose support in a bubble column bioreactor was investigated. The cells were attached to the support and grew well on the support in the bubble column bioreactor. The enzyme productivity of immobilized cells was almost the same as that given by free cells in batch culture. The enzyme was able to be reproduced by the immobilized cells in the bubble column bioreactor in repeated batch culture.

KEYWORDS: bubble column bioreactor, *Aspergillus oryzae*, α-amylase, macroporous cellulose support

## 1. Introduction

Immobilized cells have been applied to many fermentation processes. However, most of the previous works have been concerned with entrapment of cells using polymer gels, such as polyacrylamide, calcium alginate, κ-carageenan or photocross-linked resins, because of the extremely mild immobilization conditions[1]. Entrapment of cells, however, involves following problems: mass transfer limitation within the gel beads; requirement of complex and sophisticated equipment for large scale preparation of gel beads for industrial fermentations[2,3]. From the view point of mass transfer and ease with which immobilization can be achieved, immobilization by passive cell adhesion to

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surfaces seems to be preferable for cell entrapment. Recently, biodegradable macroporous cellulose support has been employed for immobilization of animal cells or bacteria[4,5].

In this present paper, the production of $\alpha$-amylase by *Aspergillus oryzae* immobilized on a macroporous cellulose support in a bubble column bioreactor was investigated. The bubble column bioreactor was chosen in preference to the conventional mechanically agitated bioreactor in order to avoid the intense shear forces generated by the impellers in the latter bioreactor.

## 2. Experimental

### 2.1 Apparatus

The experimental apparatus is shown schematically in Fig. 1. MBR-PK-1000

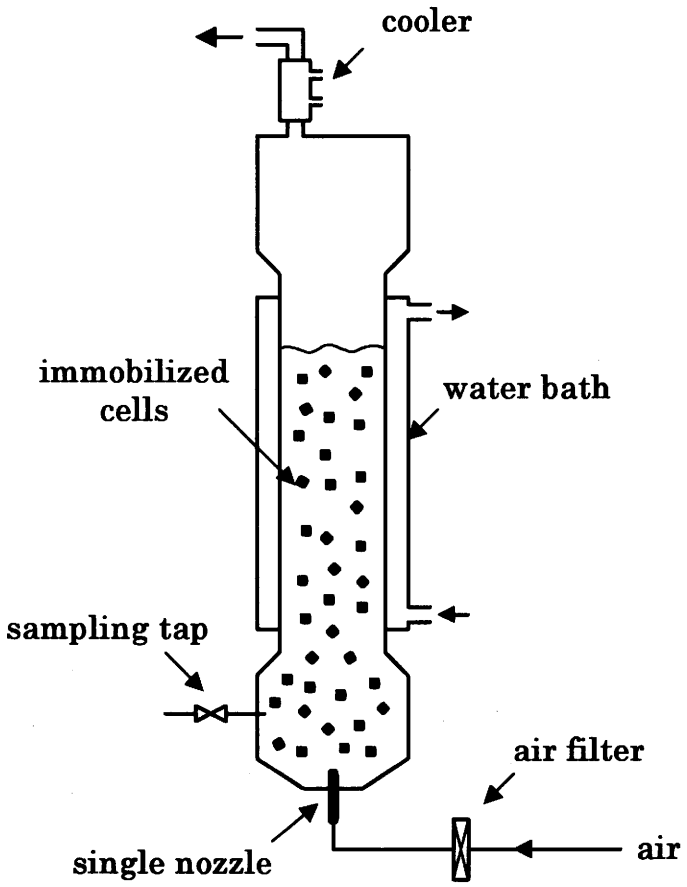


Fig. 1. Schematic diagram of experimental apparatus.

prepared by Tokyo Rika Kikai Co., Ltd., Tokyo was used for the bubble column bioreactor in the present experiment. For air sparging, a single nozzle (5 mm in diameter) was used.

2.2 Macroporous cellulose support

Cubic macroporous particles made of cellulose (Microcube BN-S03T(1000), Biomaterial Co., Ltd., Fukui, Japan) were used as a cell support material. The particle size was 3 mm.

2.3 Chemicals

All chemicals used were reagent grade purchased from Wako Pure Chemical Industries, Ltd.(Osaka, Japan) unless specifically noted otherwise.

2.4 Microorganism and medium

*Aspergillus oryzae* IFO 30113 was used as the typical α-amylase producing mold. SPY medium had the following composition: 10 g/l soluble starch, 5 g/l polypepton, 3 g/l yeast extract. The strain was maintained on SPY agar slant.

2.5 Culture conditions

The spore suspension was used for immobilization. The spores were harvested by adding 5 ml of sterilized distilled water to the slant. The spore suspension (500μl) was inoculated into 1000 ml of SPY medium with or without 1000 pieces of macroporous cellulose supports in the bubble column bioreactor, respectively. To prevent foaming, the medium contained 1000 ppm of an antifoam agent (Disfoam CC-118, Nippon Oil & Fats Co., Ltd.,Tokyo). Air was supplied at 1.0 vvm. Temperature was kept at 30℃ and pH was not controlled during the cultivation.

2.6 Analytical methods

The α-amylase activity was assayed as follows. 0.1 ml of enzyme solution was added to 0.5 ml of 0.1% soluble starch (Kanto Chemical Co., Inc., Tokyo, Japan) solution in 0.25 M phosphate buffer (pH 7.0), and the mixture was incubated at 30℃ for 5 min. Then, 0.1 ml of 3 M HCl solution was added to the mixture. 0.5 ml of 0.005 M iodine solution (Kanto Chemical Co., Inc., Tokyo, Japan) and 5 ml of distilled water were added the mixture and the mixture was measured at 660 nm. One unit of α-amylase was defined as the amount of amylase which produced 10% reduction in the intensity of blue

color of amylose-iodine complexes under the conditions mentioned above.

The total sugar concentration was determined by the phenol-sulfuric acid method[6]. Glucose concentration was determined by Glucose-Test Wako.

## 3. Results and Discussion

### 3.1 Production of α-amylase in batch culture

*Aspergillus oryzae* was able to be immobilized on the macroporous cellulose supports and grew well on the supports in the bubble column bioreactor during the cultivation. The time courses of α-amylase activity and total sugar and glucose concentration in the culture broth with or without macroporous cellulose supports in batch culture are shown in Fig. 2. It was found that both free and immobilized cells produced almost the same amount of α-amylase. The enzyme activity reached maximum values (about 38 units/ml) after 2 days incubation.

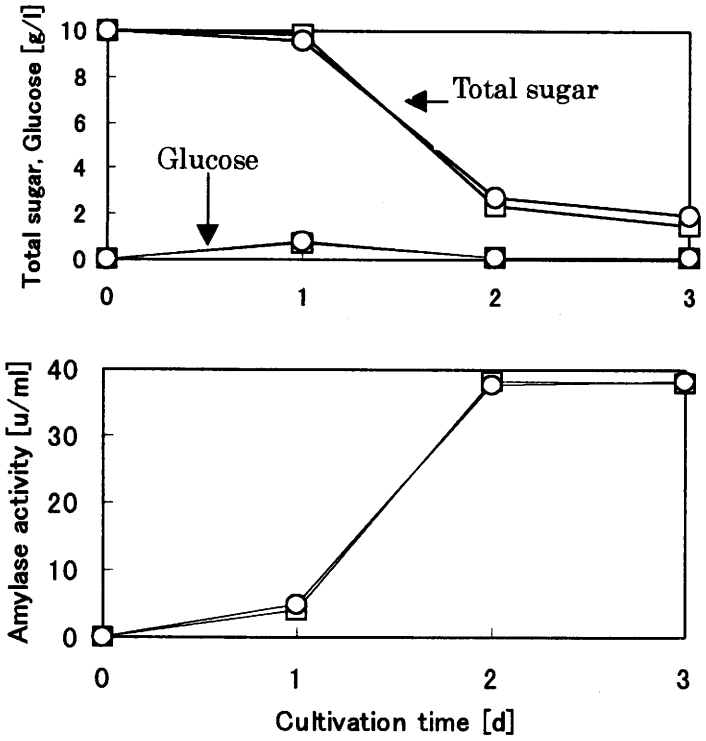


Fig. 2. Time courses of total sugar, glucose and α-amylase in batch culture.
Symbols: ○,free cells; □, immobilized cells

3.2 Production of α-amylase in repeated batch culture

The stability of α-amylase production using the cells immobilized on the macroporous cellulose supports in repeated batch culture was investigated. Initially, the cultivation was carried out in batch operation and repeated batch operation was followed after 2 days incubation. The results are shown in Fig. 3. During this period, α-amylase activity has been reproduced by the immobilized cells.

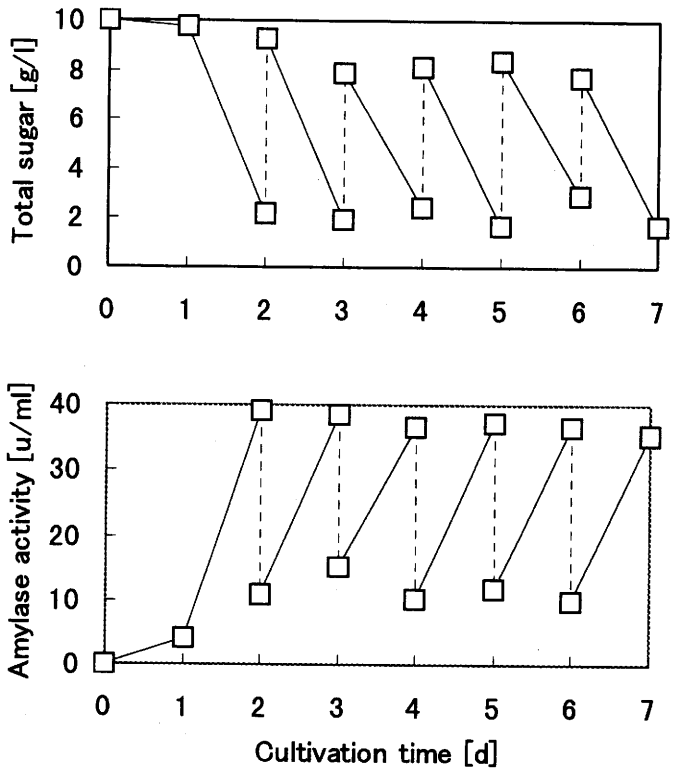


Fig. 3. Time courses of total sugar and α-amylase production in repeated batch culture. Symbol was the same as Fig. 2.

## 4. Conclusions

α-amylase was produced by *Aspergillus oryzae* immobilized on a macroporous cellulose support in a bubble column bioreactor. The enzyme was also able to be reproduced by the immobilized cells in repeated batch culture.

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