Production of Amylase by immobilized *Aspergillus oryzae* in an anaerobic vessel

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α-Amylase production by *Aspergillus oryzae* IFO 30113 immobilized on a surface of silicone tube in an anaerobic vessel was investigated. Silicone tube was used as a support material for immobilization of the mold, since silicone membrane can permeate oxygen. The cells of *A. oryzae* were adhered on the surface of the silicone tube and grew well on the support in an anaerobic vessel. α-Amylase was also produced by the immobilized *A. oryzae*.

Key words: α-amylase, *Aspergillus oryzae*, silicone membrane, oxygen

1.Introduction

Immobilized cells have been applied to many fermentation processes. However, most of the previous studies have been concerned with entrapment of cells with polymer gels, such as calcium alginate, κ-carrageenan, polyacrylamide 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 surfaces seems to be preferable for cell entrapment.

In this paper, the production of α-amylase by *Aspergillus oryzae* immobilized on a surface of silicone tube in an anaerobic vessel was investigated.

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2.Experimental

2.1 Microorganism and medium

The typical α-amylase producing microorganism, *Aspergillus oryzae* IFO 30113 was used. 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.

2.2 Culture conditions

Silicone tube (Iuchi co., Ltd., Osaka, Japan), which can permeate oxygen, was used as a support material for immobilization. The length of the tube was 2 m and the inner and outer diameter were 3 mm and 5 mm, respectively. The tube was attached in a 2.5L-aerated agitated vessel (M-100, Tokyo Rikakikai co., Ltd., Tokyo, Japan). The vessel was gassed continuously with oxygen-free N_2 (0.1 L/min) and the agitation speed was kept at 50 rpm. To increase oxygen permeation through the membrane, the air (1.0 kgf/cm^2) was supplied inside of the tube.

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 was inoculated into 1.5 L of SPY medium in the 2.5L-anaerobic vessel. Temperature was kept at 30℃ and pH was not controlled during the cutivation.

2.3 Analytical methods

The α-amylase activity was assayed as follow. 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 (pH7.0), and the mixture was incubated at 30℃ for 5 min. Then, 0.1 mL of 3 M HCl solution was added to the mixture. 0.1 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 above.

The total sugar concentration was determined by the phenol-sulfuric acid method.[4] Glucose concentration was determined by Glucose-Test Wako.

The diffusivity of oxygen through the membrane was determined by measuring the increase of the dissolved oxygen (DO) concentration in the water with an oxygen probe inserted in the vessel. Distilled water (1.5L) degassed with N_2 was placed in the vessel. The liquid was mixed in the vessel and incubated at 30℃.

3. Results and Discussion

The effect of surface roughness of silicone tube on the immobilization of cells was investigated using two types of silicon tube. The two types of silicone tube had the same thickness (1mm), but one had smooth surface and the other had rough surface facing the medium. The smooth surface of silicone tube was converted to the rough surface of silicone tube by polishing with sandpaper. Scanning electron micrograpfs of the smooth and the rough surfaces of silicone tube are shown in Fig. 1.

Photographs of growing cells on the smooth or the rough surfaces of silicone tube in an anaerobic vessel are shown in Fig. 2. The cells of *A. oryzae* were adhered on both the surfaces of silicone tube. But the growth of cells on the rough surface of silicone tube seemed better than that on the smooth surface of silicone tube. Comparing α -amylase production between these tubes, the α -amylase production on the rough surface was also better than that on the smooth surface (Fig. 3).

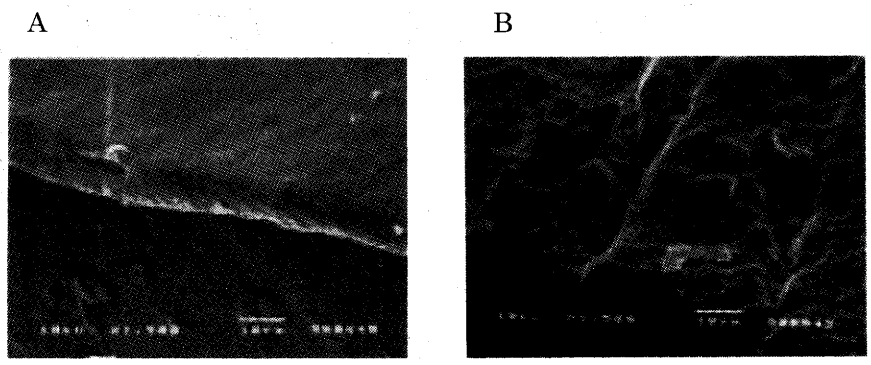

Fig. 1. Scanning electron micrograpfs of the surfaces of silicone tube.
(A) Smooth surface; (B) rough surface.

The oxygen diffusivity of the membrane was almost the same irrespective of whether the smooth surface (6.54×10^{-9} $m^2 \cdot s^{-1}$) or rough surface (6.12×10^{-9} $m^2 \cdot s^{-1}$). It was concluded that the surface condition did not affect the oxygen diffusion rate significantly and that the case of the differences in α -amylase production were due to membrane surface roughness. Thus the spores of *A. oryzae* may fall into hollows of rough surface of silicone tube and then the cells can grow well on the whole surface of silicone tube.

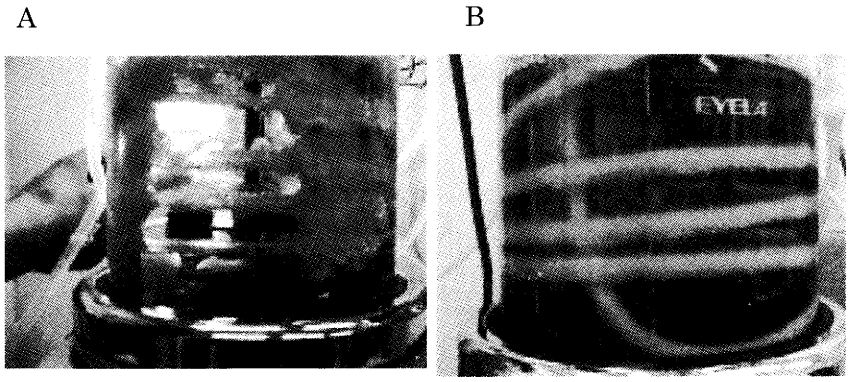

Fig. 2. Photographs of immobilized cells at 48 hr of cultivation time.
(A) Smooth surface; (B) rough surface.

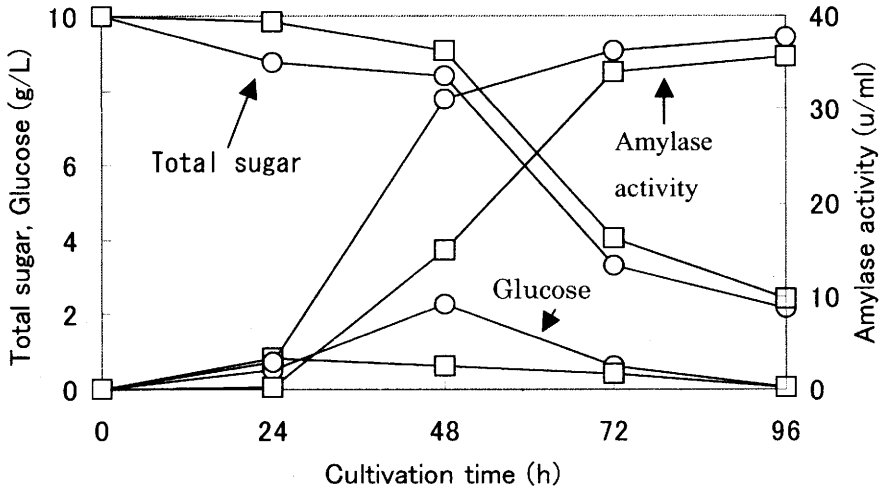

Fig. 3. Time course of α-amylase production.
Symbols: □, smooth surface; ○, rough surface.

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