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Growth of Yeast Cells Immobilized with Porous Swelling Carriers Produced by Radiation Polymerization

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Abstract

A new method of immobilization of yeast cells for the advantageous growth of cells inside and on the surface of a polymer carrier by physical adsorption is proposed. Porous and swellable polymer carriers were prepared by radiation-induced polymerization at low temperature. These polymer carriers were incubated with yeast cells at 30°C under aerobic conditions. Yeast cells were adsorbed on the surface of polymer carriers and subsequently infiltrated the polymer carriers by multiplication. The ethanol productivity of immobilized growing yeast cells thus obtained was thirteen times that of free yeast cells in a 1 : 1 volume of liquid medium to immobilized yeast cells.

Index Entries: Yeast cells, absorption on polymer carriers; growth of yeast cells, into polymer carriers; ethanol production, of immobilized yeast cells; immobilization, of yeast cells; physical adsorption of yeast cells; porous swelling polymer carrier; radiation polymerization, to produce carriers for immobilized yeast cells.

Introduction

Recently energy produced by biomass conversion (*1*) has attracted much attention.

Yeast cells have been immobilized by various methods in order to change glucose to ethanol. Wada et al. (*2*) proposed a new method for the immobilization of growing yeast cells. These authors entrapped a very small number of yeast cells in

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carrageenan matrix and the immobilized yeast cells grew in a complete medium much more densely than those in a liquid medium.

In the previous report (*3*), we reported on yeast cells that were able to multiply after immobilization by the method of γ-irradiation. First porous polymer carriers were prepared by radiation-induced polymerization at low temperature. These porous polymer carriers were immersed into the solution of precultured yeast cells to adsorb the cells. Then the porous carriers were immersed again into the monomer and irradiated to polymerize monomer. Yeast cells thus obtained showed a much higher activity than free yeast cells in the same volume of liquid medium as the volume of immobilized yeast cells.

During this investigation, the porous polymer carriers that had been shaken at 30°C with precultured yeast cells in the nutrient medium showed active ethanol production. These results were ascribed to the multiplication of yeast cells either inside or on the surface of polymer carriers under aerobic conditions.

This procedure was successful for the immobilization of yeast cells and might have great advantage for the immobilization of microbial cells. The obvious advantage of this manner of polymerization is that it has no influence on the activity of the immobilized microbial cells and is easy to handle.

In the present study, we report on multiplying yeast cells immobilized by this adsorption method.

Materials and Methods

Microorganisms

Saccharomyces formosensis was used in this study. The yeast cells were precultured under aerobic conditions for 24 h at 30°C in a medium consisting of 1% glucose, 0.1% molasses, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract (pH 4.8).

Preparation of Porous Polymer Carriers for the Immobilization of Yeast Cells by Radiation-Induced Polymerization at Low Temperature

Various compositions of glass-forming monomers, such as methoxypolyethyleneglycol methacrylate (M-23G) or 2-hydroxyethyl acrylate (HEA) were mixed with water. The mixtures were irradiated at −78°C with γ-rays from a ^{60}Co source for 1 h with a dose rate of 1×10^6 rad/h. In some cases, glucose or K_2HPO_4 were added to the mixture. The resultant polymer carriers were cut into small pieces, approximately 2–3 mm in diameter, and shaken with excess amount of water for 3 d. These polymer carriers were sterilized by autoclaving at 120°C for 40 min.

Immobilization of Yeast Cells with Porous Polymer Carriers Produced by Radiation-Induced Polymerization at Low Temperature

Porous polymer carriers thus obtained were immersed in the mixture of precultured yeast cells and nutrient medium. The obtained suspension was incubated at 30°C under aerobic conditions in a rotary shaker. The composition of the nutrient me-

dium was 11.5% glucose, 1% molasses, 0.15% yeast extract, 0.25% NH_4Cl, 0.1% NaCl, 0.001% $CaCl_2$, and 0.3% lactic acid (pH 4.8).

Analytical Method

Ethanol produced was determined by using alcohol dehydrogenase (*4*).

Results

Porous polymer carriers produced by radiation-induced polymerization at low temperature were cut into small pieces and shaken with an excess amount of water for 3 d. Some polymer carriers swelled remarkably and the volume of polymer carrier increased 5–10 times in volume by swelling. After sterilization in an autoclave, these porous polymer carriers were immersed in the mixture of precultured yeast cells and the nutrient medium. The resultant suspension was incubated at 30°C under aerobic conditions on a rotary shaker. Every 24 h, the nutrient medium was changed.

Many colonies of yeast cells were observed after aerobic incubation at 30°C for 24 h on the surface of porous polymer carriers. When these samples were incubated further under aerobic conditions, the layer of yeast cells covered the whole surface of the polymer carriers. The thickness of the yeast cell layer increased and reached 2–3 mm after aerobic incubation for 100 h. In Fig. 1, the layer of dense yeast cells can be seen inside, but near the surface, of the polymer carriers.

After aerobic incubation for various periods (A), polymer carriers with entrapped yeast cells were picked out and washed well with nutrient medium. The washed immobilized growing yeast cells (wet weight: 2 g) were put into nutrient medium (volume ratio of immobilized cells to medium, 1 : 1) and fermented by incubation at 30°C under gentle rotary shaking for 1 h in order to test alcohol productivity. After a 1 h fermentation, the concentration of alcohol produced (B) was measured. The relation between the ethanol concentration after 1 h anaerobic fermentation at 30°C (B) and the period of aerobic incubation (A) was investigated, and the results are shown in Fig. 2.

Without aerobic incubation of immobilized yeast cells, the ethanol concentration after fermentation for 1 h was very low (see Fig. 2). The ethanol concentration after fermentation for 1 h (B) increased rapidly with the increase in the aerobic incubation period (A), as shown in Fig. 2. After aerobic incubation from 72 to 120 h, the ethanol concentration after 1 h of fermentation reached 2.9–3.8%, although results were different depending upon the composition of the carriers (see Fig. 2). During fermentation for 1 h, violent evolution of CO_2 gas was observed on the surface of polymer carriers.

Some immobilized yeast cells retained high ethanol producing activity even after aerobic incubation for 480 h, and gave more than 3.5% ethanol concentration after a 1 h fermentation (see Fig. 2).

As a control on cell growth, the same number of free cells were transferred into a sterilized Erlenmeyer flask containing the nutrient medium and incubated under the same conditions as a like number of immobilized cells.

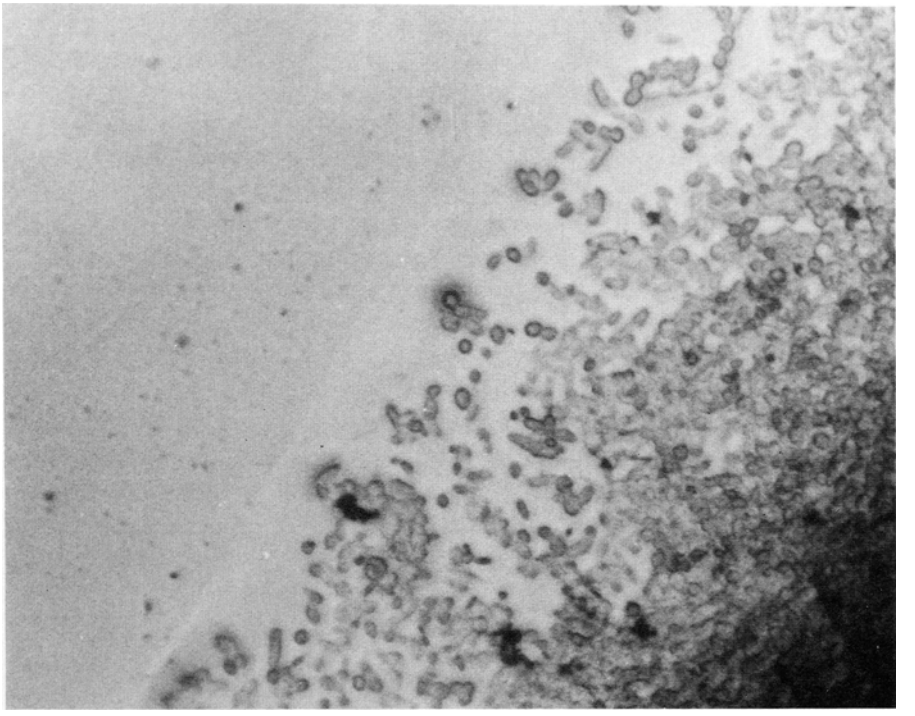

Fig. 1. Microphotograph of the section of polymer carrier with immobilized growing yeast cells incubated at 30°C under aerobic conditions for 100 h (×100). The right side of the photograph is the inner part of matrix immobilizing yeast cells.

After around 70 h of aerobic incubation, the concentration of alcohol after a 1 h fermentation in this free system reached a maximum of about 0.3%, as shown in Fig. 2.

In the case of alcohol production tests for immobilized and free yeast cells described above, the fermentation reactions were stopped at 1 h and the alcohol concentrations were measured. The whole fermentation, with complete conversion of glucose and molasses, was then carried out and the results are shown in Fig. 3. The immobilized yeast cells were incubated by gentle shaking with nutrient medium (volume ratio of immobilized cells to medium, 1 : 1). As shown in Fig. 3, the ethanol yield reached up to 100% within 150 min. In the free system, the ethanol yield reached up to 13% within 150 min. This result with immobilized yeast cells corresponds the ethanol productivity of 20 mg/mL of gel/h^2.

Discussion

Densely packed yeast cells can be seen inside the boundary that seems to be the surface of the polymer carrier in the microphotograph of the section of the carrier shown in Fig. 1. Near the boundary, isolated yeast cells are stably enclosed by surrounding matrix. These immobilized yeast cells were thrown into water and shaken vigorously, and few yeast cells were released.

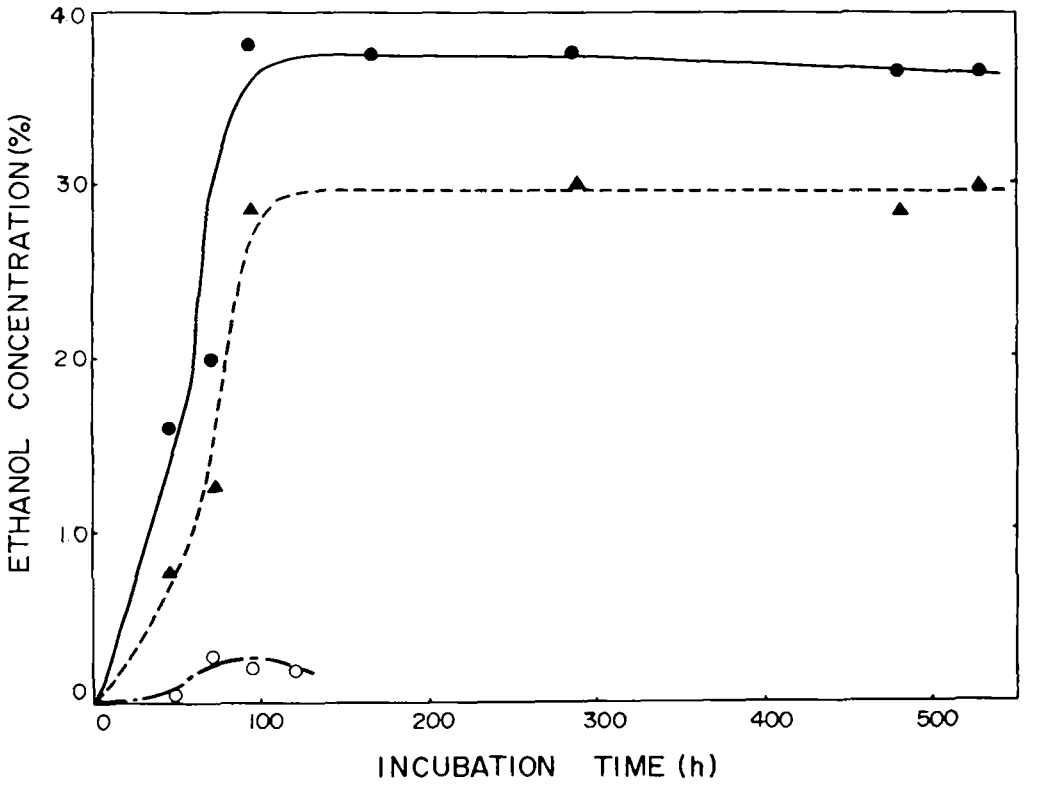

Fig. 2. The change of ethanol concentration after a 1 h fermentaion in immobilized growing yeast cell system. Porous polymer carriers produced by radiation-induced polymerization at low temperature were shaken with water. Swollen polymer carriers were shaken with precultured yeast cells and the nutrient medium at 30°C under aerobic conditions for various periods. Nutrient was changed every 24 h. Immobilized yeast cells were washed well with nutrient mediem. A 1-h fermentation reaction was carried out by using these immobilized yeast cells, and ethanol concentration was measured. The relation between aerobic incubation time and ethanol concentration is shown in this figure. As a control, free intact cells were incubated aerobically for various periods. Alcohol concentration after a 1-h fermentation reaction was measured under precisely the same conditions as immobilized yeast cells.

	Components of Carriers		
	M-23G	HEA	Water
●	1	1	4
▲	1	1	2
○	free cell		

These results indicate that yeast cells infiltrated the interior of polymer carriers during aerobic cultivation at 30°C in nutrient medium.

We suggest that the mechanism of intrusion or infiltration of yeast cells is as follows: First, some yeast cells adsorb on the surface of polymer carriers, especially on a dent or fold of the surface. Second, the adsorbed yeast cells intrude or infiltrate into the interior of the polymer carriers through small pores. Third, the yeast cells that have gotten inside the polymer carriers multiply briskly. The density of yeast

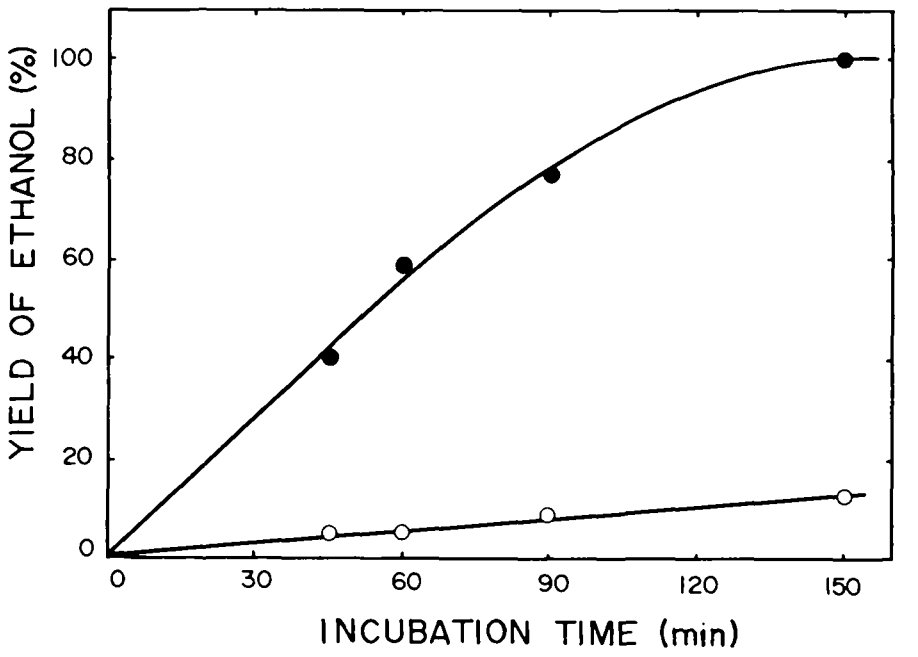

Fig. 3. Time conversion curve of the fermentation reaction with immobilized and with free yeast cells. A 6.25% ethanol concentrtation means 100% conversion of 11.5% glucose and 1% molasses: (●) Immobilized yeast cells; yeat cells immobilized and incubated aerobically for 72h; Components of carriers for immobilization: M-23G, HEA 1, water 2. (○) Free yeast cells.

cells inside the polymer carriers then increases rapidly and becomes much greater than the density of yeast cells that are not immobilized in the liquid nutrient medium. The increased volume of yeast cells from this multiplication results in the extension of surrounding polymer carriers. This progressive multiplication of yeast cells can explain the continuous distribution of yeast cells within the polymer carriers shown in Fig. 1.

There have been many investigations on the immobilization of yeast cells. Most immobilization studies have adopted the entrapping method (*2*) and some others have used the physical adsorption method (*5*). Almost all studies on the immobilization of yeast cells have treated yeast cells without multiplication; only a few studies (*2*) have dealt with yeast cells and their multiplication. The present study employed a physical adsorption method that was accompanied by yeast cell multiplication.

Many entrapping methods are not biologically inert and cause damage to cells during the immobilization process. Physical adsorption methods have no disadvantages of this type. Almost all studies that adopted physical adsorption methods used hard carriers such as synthetic resins (*5*). These hard carriers are not very suitable for yeast cell multiplication because they cannot change their shape correspondingly with the increasing volume of yeast cells as they multiply inside the carriers. In contrast, the porous swelling polymer carriers used in the present study are very soft and elastic, and can readily change their shapes correspondingly with

the increasing volume of yeast cells that multiply inside them. We believe this receptivity of carriers—their ability to change shape and accept the increased volume of yeast cells—is very important for the multiplication of immobilized yeast cells.

In the present study, the yeast cells that had entered the carriers were found in layers near the surface of the polymer carriers, as shown in Fig. 1. The substrate of the fermentation reaction can thus easily make contact with the yeast cells immobilized near the surface of the carriers. With many methods for immobilizing microbial cells or enzymes, the performance of the immobilized enzymes is poor because of diffusion limitation. In the present study, this negative effect is avoided because yeast cells are immobilized near the surface of polymer carriers. Moreover, the polymer carriers have a highly porous structure. The substrate can thus penetrate inside the polymer carriers and can readily make contact with yeast cells immobilized inside of carriers.

In the present study, the activity of immobilized yeast cells was thirteen times that of cells in a free system. This amplification is much better than in our previous report (*6*), where the activity of yeast cells immobilized by radiation-induced polymerization of glass-forming monomers with hydrophilic filler was only three times that of cells in a free system. This result indicated that porous swelling polymer carriers produced by radiation-induced polymerization at low temperature are suitable for immobilization and multiplication of yeast cells. The magnification of activity in the present study is slightly greater than in the report of Wada et al. (*2*), where the activity of yeast cells immobilized with carrageenan was ten times that of cells in a free system, and has been the highest absolute activity of all hitherto known studies on the use of immobilized yeast cells. However, the rate of fermentation reaction by immobilized yeast cells in the present study was smaller than that in their report. In our study, 100% ethanol conversion was obtained within 150 min, as shown in Fig. 3. The same conversion was obtained within 60 min in their report. This difference might result from the absolute activity of free yeast cells in the present study being smaller than that of the free yeast cells in their report.

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