Nature of Yeast Cells Immobilized by Radiation Polymerization Activity Dependence on the Molecular Motion of Polymer Carriers

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Higher activity of ethanol production due to higher density of yeast cells, was observed in yeast cells immobilized with artificial polymer carriers of higher molecular motion. These polymer carriers were prepared by radiation-induced polymerization below 0 °C. Yeast cells were immobilized with these carriers by adsorption method during multiplication. Molecular motion of polymer carriers in swollen state was evaluated by the line width of nuclear magnetic resonance spectrum. A possible reason for higher activity was discussed. This study of relation between molecular motion of polymer carriers and activity of yeast cells immobilized with these carriers, is very useful to design the chemical and physical structure of polymer carriers for immobilization of microorganisms.

Introduction

Many methods for immobilizing microbial cells have been reported with natural [1, 2] and artificial carriers [3].

We have studied a few methods to immobilize yeast cells with artificial polymer carriers produced by radiation polymerization [4, 5]. Recently, we have proposed a new method of immobilization of yeast cells for the advantageous growth of cells inside and on the surface of an artificial polymer carrier by physical adsorption [6]. Porous swelling polymer carriers produced by radiation polymerization were incubated aerobically with precultured yeast cell in nutrient medium. The immobilized yeast cells thus obtained exhibited activity of around ten times that of cells in free system. This greater activity was due to much higher density of yeast cells trapped inside and on the surface of polymer carriers [6].

Radiation polymerization can produce artificial polymer carriers for immobilizing yeast cells, various kinds of nature of which can be changed contineously. We have studied the relation between the nature of these carriers and the activity of yeast cells immobilized with these carriers [7]. These studies would give important suggestions how to immobilize not only yeast cells but also other microbial cells.

We have already studied the effect of water content of polymer carriers on the activity of yeast cells immobilized with these carriers [7]. Polymer carriers of various water content were prepared by radiation polymerization of monomers of various concentration. Yeast cells immobilized with carriers of higher water content exhibited higher activity, so far as the carriers had mechanical strength enough to withstand shaking during aerobical incubation.

These results was interpreted as follows. Polymer carriers of higher water content have more space to keep more nutrient medium inside carriers at the initial stage of aerobical incubation. More nutrient medium inside carriers could be changed with more yeast cells with progress of aerobical incubation.

This interpretation explains a part of, but not of all of, higher activity of immobilized yeast cells. Factors of polymer carriers other than water content should be introduced. As one of these factors, we introduce here molecular motion of polymer carriers in swollen state evaluated with high-resolution NMR [8]. We will describe the effect of molecular motion of carriers on the activity of yeast cells immobilized with these carriers.

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% pepton, 0.3% yeast extract, and 0.3% malt extract (pH 4.8).

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Preparation of swelling polymer carriers for the immobilization of yeast cells by radiation-induced polymerization below 0 °C

Various concentrations of two kind of glass-forming monomers, methoxypolyethyleneglycol methacrylate (M-23G) and 2-hydroxy-ethylacrylate (HEA) were mixed with water.

The mixture were irradiated at −78 °C with γ-rays from a ^{60}Co source for 1 h with a dose rate of 1×10^6rad/h. 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 in order to be fully swollen. These fully swollen polymer carriers were sterilized by autoclaving at 120 °C for 40 min.

When these carriers were incubated with yeast cells aerobically in nutrient medium at 30 °C, 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 72 h. Yeast cells were immobilized on the surface and inside of polymer carriers [6]. The composition of the nutrient medium used in this study was 12% 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).

Evaluation of activity in immobilized yeast cells

After aerobic incubation for 72 h, polymer carriers with entrapped yeast cells were washed well with nutrient medium. The washed immobilized growing yeast cells (wet weight: 10 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. After the fermentation for 1 h, the concentration of ethanol was determined. This concentration of ethanol was used as the indication of activity in immobilized yeast cells.

Evaluation of the degree of molecular motion in the swelling polymer carriers by high-resolution NMR

The polymer carriers were fully swollen with H_2O as described. In order to avoid the overlapping large signal of H due to H_2O in high resolution spectrum, H_2O inside polymer carriers were replaced with D_2O, as follows. Polymer carriers fully swollen with H_2O, were lyophilized for 72 h. The lyophilized polymer carriers were shaken with excess amount of D_2O for 4 d in order to be fully swollen with D_2O. These polymer carriers fully swollen with D_2O were used for evaluation of the degree of molecular motion by high-resolution NMR [8]. High-resolution FT-MNR spectra were recorded by a JEOL PS-100 spectrometer operating at 100 MHz.

Determination of ethanol concentration

Ethanol produced was determined by using alcohol dehydrogenase [9].

Results and Discussion

To prepare polymer carriers of various property, various concentration of monomers (HEA, M-23G 1:1) were mixed with water for radiation-induced polymerization. The fermentation reaction was carried out with these immobilized yeast cells of various property. The ethanol productivity was evaluated by the concentration of ethanol after the fermentation for 1 h.

Just before immobilization, fully swollen polymer carriers were divided into two parts. One part was used for immobilization. The other part was used for evaluation of molecular motion and water content of polymer carriers prepared with monomers of various concentration. The lower concentration of monomers gave higher value of molecular motion and water content in this polymer system.

The ethanol productivity of yeast cells immobilized with various polymer carriers prepared with monomers of various concentration was plotted against the water content of polymer carriers [7]. In this case, the monomer concentration before polymerization to produce polymer carriers was between 14 and 89%. When the water content of polymer carriers increased, the ethanol productivity increased. The maximum ethanol productivity was obtained with polymer carriers where the water content was 94.4% and total initial monomer concentration before polymerization was 29%.

As the water content of polymer carriers increased, the ethanol productivity increased in the yeast cells immobilized with these polymer carriers. One reason of these results was considered as follows [7].

In this study, the polymer carriers polymerized by radiation-induced polymerization, were shaken with large amount of water, in order to be swollen as much as possible. The swollen polymer carriers were immersed into the nutrient medium containing yeast

cells. The yeast cells were adsorbed on the surface of polymer carriers and gradually immobilized. At the early stage of immobilization, the polymer carriers were swollen with nutrient medium as much as possible. This nutrient medium inside of polymer carriers must be replaced with yeast cells with the proceeding of immobilization process.

Higher water content of polymer carriers permit the presence of more amount of nutrient medium inside of the polymer carriers, which swell the polymer carriers. This situation provides more space where yeast cells can intrude and live inside the polymer carriers.

These consideration could partly explain the higher ethanol productivity in yeast cells immobilized with polymer carriers of higher water content. However we could not explain with above reason only. In the present study, water content of polymer carriers increased from 75% up to 95%. The difference was only 20%. As the same time, the ethanol productivity increased twice from 20 up to 40 mg/ml of gel/hr [7]. The ethanol productivity of immobilized yeast cells is roughly proportional to the number of yeast cells trapped.

These results obviously indicate that higher ethanol productivity could not be explained only with more space inside polymer carriers where yeast cells are exchanged and cultivated. In these immobilized yeast cells of higher activity, polymer carriers must have not only more space inside them but also have some other physical properties.

Most possible physical property is the elasticity of swollen polymer carriers as we suggested previously [6]. To evaluate this elastic property of polymers, we could introduce the parameter of molecular motion. Higher molecular motion would occur in more elastic polymer matrix. Molecular motion could be evaluated by the line width of high-resolution nuclear magnetic resonance (NMR) spectrum in swollen state [8].

Fig. 1 shows the high-resolution NMR spectrum of polymer carriers fully swollen with D_2O. The marked main peak is due to the main chain of polymers, $(O-CH_2-CH_2)_n$. The degree of molecular motion of polymer carriers is inversely proportional to the NMR line width. The higher molecular motion corresponds to the narrower NMR line width [8]. The line widths of this main peak for various polymers were plotted against the water content of polymers and shown in Fig. 2. As the water content increased, the line width of NMR main peak decreased monofonically.

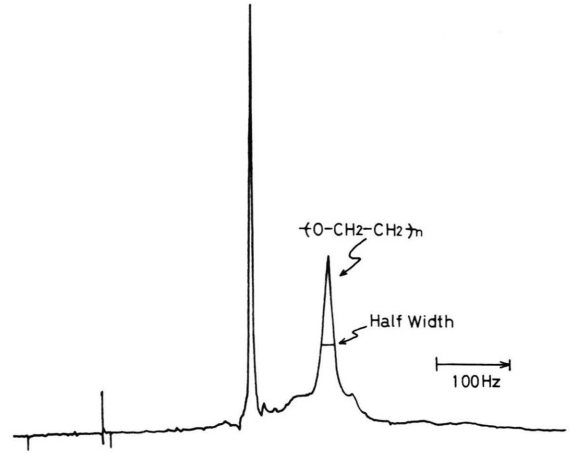

Fig. 1. High-resolution NMR spectrum obtained with polymer carriers fully swollen with D_2O. The marked main peak is due to the main chain of polymers, $(O-CH_2-CH_2)_n$. The sharp peak without mark is due to residual H_2O.

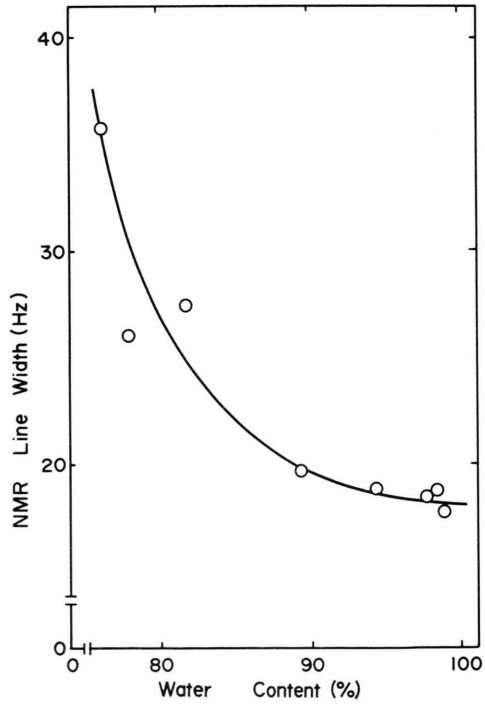

Fig. 2. Dependence of NMR line width on the water content of polymer carriers with which yeast cells were immobilized. Water content was varied with the concentration of monomers with which polymer carriers were produced (see text).

The line width of NMR main peak for various polymer carriers were plotted against the ethanol

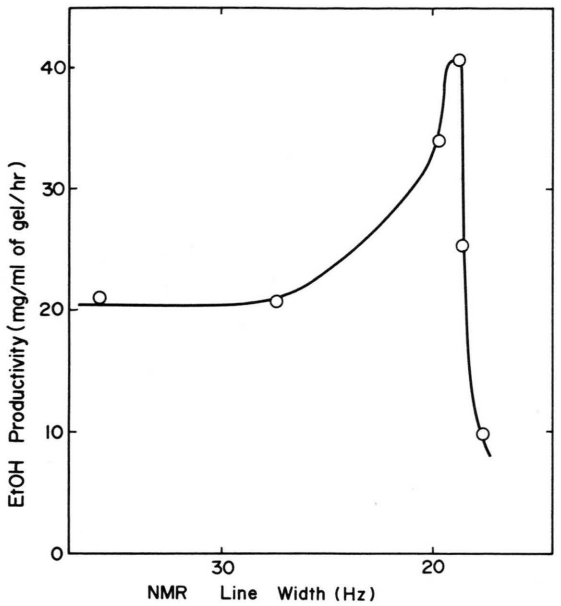

Fig. 3. Dependence of ethanol production activity on the water content of polymers with which yeast cells were immobilized (see text).

productivity of yeast cells immobilized with these polymer carriers and shown in Fig. 3. As the line width decreased, the ethanol productivity increased. Consequently, as the molecular motion of polymer carriers increased, the ethanol productivity increased.

These experimental results could be explained as follows. During aerobic incubation of polymer carriers with yeast cells in nutrient medium, some yeast cells adsorbed on the surface of polymer carriers [6, 7]. The adsorbed yeast cells intrude or infiltrate into the interior of the polymer carriers through small pores [6, 7].

The circumstances could be considered as follows, where one yeast cell is surrounded by polymer carriers. The multiplication of this yeast cell requires the space for multiplicated yeast cells. Yeast cells surrounded by polymer carriers with higher molecular motion, *i.e.*, more elastic polymer carriers, could exclude surrounding polymer carriers easily by the increase of volume in yeast cells which multiply. Contrary, yeast cells surrounded by polymer carriers with lower molecular motion, *i.e.*, less elastic polymer carriers could not exclude surrounding polymer carriers easily.

In Fig. 3., abrupt drop of ethanol productivity was observed for highest molecular motion. In this case, the polymer carriers were broken into small pieces when these polymer carriers were shaken in a rotary shaker for incubation of yeast cells immobilized with these carriers. The decrease of monomers concentration results in the decrease of mechanical strength of polymer carriers polymerized with these monomers. Finally, polymer carriers became too weak to entrap yeast cells. Thus, the ethanol productivity of yeast cells immobilized with these polymer carriers decreased.

Conclusions

Yeast cells immobilized with polymer carriers of higher molecular motion exhibited higher activity of ethanol production. Yeast cells inside polymer carriers multiply excluding surrounding polymer carriers. Yeast cells surrounded by polymer carriers with higher molecular motion could exclude polymer carriers easily. This situation results in the multiplication of yeast cells more densely and the higher ethanol production activity.

In this work, the relation between molecular motion of polymer carriers and activity of yeast cells immobilized with these carriers was studied. These studies are obviously very useful to design the chemical and the physical structure of polymer carriers for immobilization of microorganisms.

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