Immobilization of Microbial Cells on Cellulose–Polymer Surfaces by Radiation Polymerization

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Synopsis

Streptomyces phaeochromogenes cells were immobilized on cellulose–polymer surfaces by radiation polymerization using hydrophilic monomers and paper. The enzyme activity of immobilized cell sheets was higher than that of immobilized cell composites obtained by the usual radiation polymerization technique. The enzyme activity of the sheets was affected by monomer concentration, the thickness of paper, and the degree of polymerization of paper. The copolymerization of hydroxyethyl methacrylate and methoxytetraethyleneglycol methacrylate in the sheets led to a further increase of the enzyme activity due to the increase of the hydrophilicity of the polymer matrix. The Michaelis constant of the sheets from low monomer concentration was close to that of intact cells.

INTRODUCTION

Industrially important immobilized microbial cells have attracted considerable interest in recent years. Practically, immobilized cell systems have been applied to industrial production of useful compounds.[1] In general, hydrophilic gels such as natural and synthetic polymers have been employed for immobilization of cells.[1–10] In immobilized cells resulting from an entrapping method, the diffusion of substrate in polymer matrix is a very important subject to induce a desired enzyme reaction. It is expected that the enzyme activity of immobilized cells is dependent on the nature of the polymer matrix. The study of the relationship between the enzyme activity and the state of the polymer matrix in immobilized cells has not been carried out. To improve the contact of cells and substrate, the immobilization of cells on the solid surfaces is reasonable. Recently, covalently linked cell fixing on the surface of the matrix has been tried.[10]

In this work, the immobilization of microbial cells such as *Streptomyces phaeochromogenes* on cellulose–polymer surfaces was studied by radiation polymerization using hydrophilic monomers and papers.

MATERIALS AND METHODS

Materials

Streptomyces phaeochromogenes cells containing active glucose isomerase were obtained from Nagase Sangyo Co., Ltd. Hydroxyethyl methacrylate (HEMA), methoxytetraethyleneglycol methacrylate (M-4G), and diethyleneglycol diacrylate (2G) were obtained from Shinnakamura Chemical Industry Co.,

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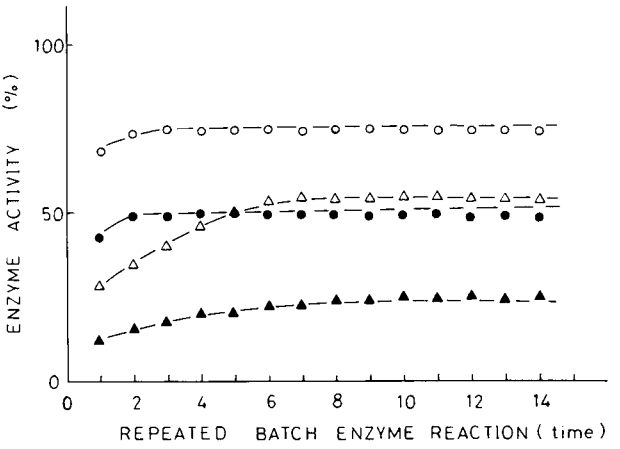

Fig. 1. Variation of the enzyme activity with repeated batch enzyme reaction. HEMA monomer concentration: immobilized cell sheets: (○) 30%; (●) 70%; immobilized cell composites: (△) 30%; (▲) 70%.

Ltd. Paper (0.05–1.0 mm thickness) used as a base material of the sheet was obtained from Toyo Roshi and the Fukuko Paper Mill Co., Ltd. Cellulose acetate (acetyl content 39.9%) was obtained from the Kanto Chemical Co., Ltd.

Preparation of Immobilized Cell Sheets

A mass of dried cells was suspended in 0.05*M* phosphate buffer (0.01*M* $MgSO_4 7H_2O$, pH 7.2) containing monomer. This solution (0.5 mL) was coated on the paper (5 × 5 cm) with a syringe; then the coated paper was put into a vessel and was irradiated in N_2 gas atmosphere at −78°C by γ-rays from a ^{60}Co source. After irradiation, immobilized cell sheets obtained were cut into a small size (1 × 1 cm) and employed for enzyme reaction.

The immobilized cell sheets from a cellulose acetate film were obtained in a similar coating method, in which cellulose acetate film (0.1 mm thickness) was obtained by a dry method of the solution (5%) in which cellulose acetate was dissolved into acetone.

Enzyme Activity

The durability of the enzyme (glucose isomerase) activity of immobilized cell sheets was examined by repeating the batch enzyme reaction (1.0 h at 65°C). The enzyme activity (%) was obtained from the D-fructose formation ratio in immobilized and intact cells with each batch enzyme reaction. The enzyme activity of the cells was evaluated from the initial reaction rate, which was found to be linear during the reaction time of the assay. A 1.0% D-glucose solution (0.05*M* phosphate buffer solution containing 0.01*M* $MgSO_4 7H_2O$, pH 7.2) was used as the substrate. The D-fructose formed was determined by the cystein–carbazole method.[11]

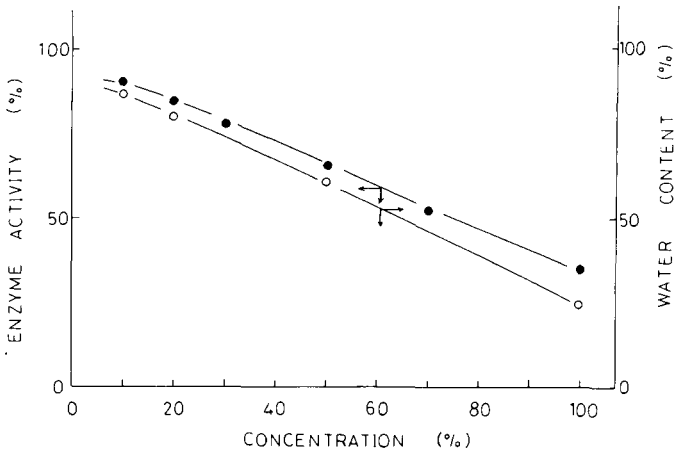

Fig. 2. Effect of HEMA monomer concentration on the enzyme activity and water content: (●) enzyme activity; (○) water content.

RESULTS AND DISCUSSION

Variation of Enzyme Activity with Repeated Batch Enzyme Reaction

The variation of the enzyme activity of immobilized cell sheets with repeated batch enzyme reactions was studied. The relationship between enzyme activity and repeated batch enzyme reactions in immobilized cell sheets is shown in Figure 1, together with that in immobilized cell composites obtained by our previous method.[12] The enzyme activities of the sheets from 30% and 70% monomer concentration had a saturated high value at the initial stage of the repeated batch enzyme reactions, but those of the composites increased gradually with repeating batch enzyme reactions. The enzyme activities of the sheets at the later stage were higher than those of the composites. Thus, a remarkable difference in the enzyme activity was observed between the sheets and composites. The immobilized cell composites obtained by radiation polymerization of hydrophilic HEMA monomer gave a relatively large block form such as a pellet form (1 mm thickness × 8 mm ϕ diameter), because the form of the composites was regulated by a cutting operation with a cryostat. Therefore, the diffusion of the substrate and product was affected by the thickness of the polymer matrix in the composites. In fact, as seen in Figure 1, the enzyme activity of the composites was lower, and the increased phenomena of the enzyme activity at the initial stage were observed, indicating that the diffusion rate of the substrate increases by swelling of the polymer matrix in water and then the enzyme reaction begins to proceed. On the other hand, the immobilized cell sheets, which were obtained by the coating method of the monomer solution containing cells, consist of the cells and the polymer matrix of thin layer coated on the cellulose fibrils of paper, so that the diffusion resistance of the substrate and product was improved. Accordingly, as can be seen in Figure 1, the enzyme activity of the sheets from 30% monomer concentration gave a high value throughout repeated batch enzyme reactions. The high enzyme activity of the sheets at the initial stage

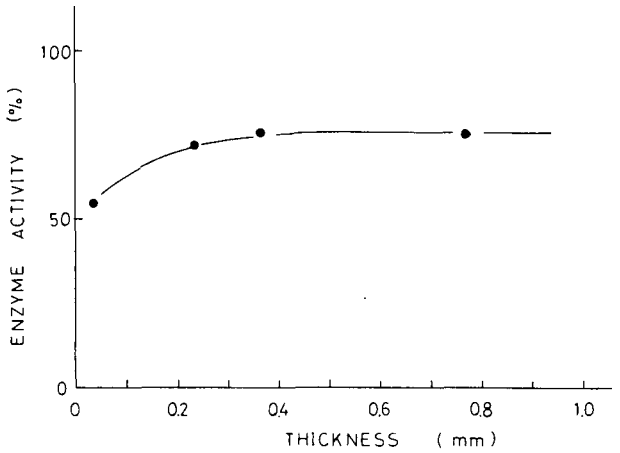

Fig. 3. Effect of the thickness of paper on the enzyme activity. HEMA monomer concentration, 30%.

indicates that the cells are trapped on near the surface of the cellulose fibrils in which the polymer matrix plays a role of an adhesion agent between the cells and cellulose fibrils.

Effect of Monomer Concentration

The effect of monomer concentration on the enzyme activity in the sheets was studied. The relationship between monomer concentration and enzyme activity or water content of the sheets is shown in Figure 2. The enzyme activity and water content decreased with increasing monomer concentration. As monomer concentration increases, the cells are entrapped into the polymer matrix, so that the enzyme reaction would be restricted. The Michaelis constant (K_m) of immobilized cell sheets from 20% monomer concentration was found to be 0.37M by Lineweaver–Burk plots, and it was close to that (0.33M) of intact cells. Furthermore, as seen in Figure 2, the enzyme activity at low monomer concentrations gave a very high value and was close to that of intact cells, indicating that the cells are trapped to be a free state like intact cells by the polymer matrix of small amounts on the cellulose fibrils, in which the diffusion of the substrate and product is not restricted. The polymer matrix on the sheets had a porous structure which is formed by the melting of ice, for example, the matrix from 10% monomer concentration should be almost occupied by the pore of water phase (90%) and the cells, in which the size of the pore is 1–5 μm. This pore structure in the polymer matrix led to the further increase of the enzyme activity at low monomer concentrations.

Effect of the Thickness of Paper

The relationship between the thickness of paper and enzyme activity is shown in Figure 3. The enzyme activity of the sheets increased slightly with increasing the thickness of paper. The decrease of the enzyme activity at low thickness

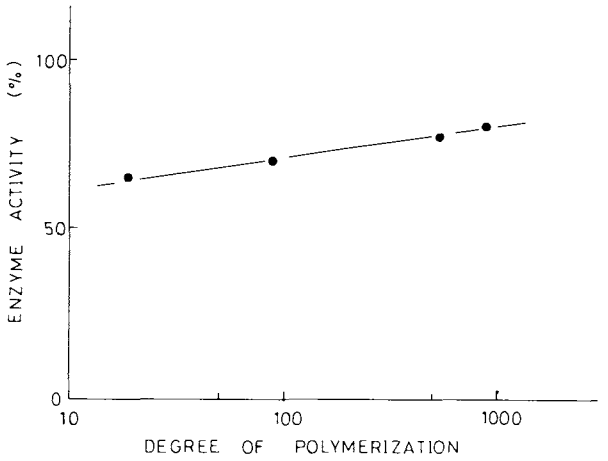

Fig. 4. Effect of the degree of polymerization of paper on the enzyme activity (the degree of polymerization of paper was regulated by radiation treatment method in Ref. 13).

would be due to the increase of the thickness of the polymer matrix layer coated on the cellulose fibrils because the amount of the coated monomer solution was constant. As can be seen in Figure 3, the enzyme activity leveled off at a high thickness of paper, and this is probably due to the fact that the cells are not dispersed into paper and are localized on the surface of paper, though small amounts of the cells are dispersed into paper to trap with the polymer matrix. Thus, paper having about 0.5 mm thickness appeared to be suitable for the immobilization, and the sheets obtained from a 0.5 mm thickness of paper having a certain mechanical strength seem to be useful for the bioreactor of various types.

Effect of the Degree of Polymerization

The effect of the degree of polymerization of the cellulose fibrils of paper on the enzyme activity was studied. Figure 4 shows the relationship between enzyme activity and degree of polymerization. The enzyme activity decreased with decreasing the degree of polymerization. The sheets from papers with a low degree of polymerization gave a firmly compact state consisting of the polymer matrix and the cellulose fibrils of short lengths. The cellulose fibrils of the papers with a low degree of polymerization, which are obtained by the radiation degradation treatment, had a weak mechanical strength resulting from the degradation of cellulose molecular chains. The sheets obtained from such a paper appeared to be a composite state, in which the polymer matrix is probably invaded into the degraded cellulose fibrils and then the cells and the cellulose fibrils are compactly hardened. The sheets from the papers with a high degree of polymerization gave a flexible and soft property, having a reasonable mechanical strength. From these results, it was found that the papers with a high degree of polymerization are favorable as a base material for the immobilization of cells.

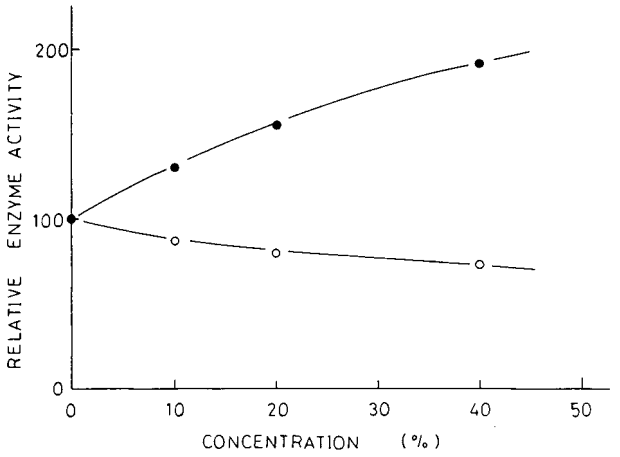

Fig. 5. Effect of the addition of 2G and M-4G monomer on the enzyme activity. Total monomer concentration, 50%; monomer: (○) 2G; (●) M-4G

Effect of the Addition of Other Monomers

The effect of the addition of hydrophilic (M-4G) and hydrophobic (2G) monomers in the present method, using HEMA monomer was studied. The relationship between relative enzyme activity and addition concentration is shown in Figure 5. The enzyme activity in the addition of 2G monomer decreased, but that in HEA monomer increased with increasing addition concentration. The sheets in the addition of HEA monomer gave a soft state due to the increase of hydrophilicity. The water content which shows a hydrophilicity of the polymer of the M-4G monomer was 75%, and this was very much larger than that (26%) of the HEMA monomer. The copolymerization of such a high hydrophilic monomer with HEMA monomer changed the property of the polymer matrix to be a more porous gel, in which the cells would be trapped on the cellulose fibrils in a relaxed state. It is reasonable to consider that this change of the polymer matrix in the sheets led to the increase of the enzyme activity, taking a release of the diffusion resistance of substrate, while the sheets from the copolymerization of 2G and HEMA monomer were a relatively rigid state in comparison with those from only HEMA monomer, owing to the effect of crosslinking of bifunctional 2G monomer, in which the cells were fixed in very firm state. The immobilization of the cells in the firm state appeared to be undesirable, due to the restriction of the enzyme reaction in the polymer matrix.

Immobilization of the Cells with Cellulose Acetate Film

The cells were immobilized using cellulose acetate film instead of paper as a base material, and the effect of base material in the present method was studied. The relationship between enzyme activity and repeated batch enzyme reaction is shown in Figure 6. The enzyme activity decreased first and became constant with repeated batch enzyme reaction. The enzyme activity in the sheets from cellulose acetate was lower than that from paper. This difference is due to the difference of the structure of the base material between cellulose acetate film

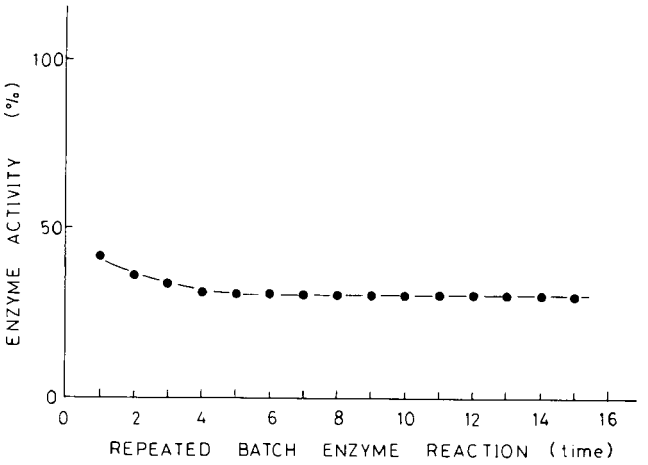

Fig. 6. Variation of the enzyme activity with repeated batch enzyme reaction in the sheets from cellulose acetate film. HEMA monomer concentration, 50%.

and paper. The surface area of the sheets from paper is larger than that from cellulose acetate film, because paper consists of a network structure of cellulose fibrils and the cells are trapped on its surface. In the sheets from paper, it is thought that the polymer matrix is grafted to the cellulose fibrils by radiation reaction. In fact, the polymer matrix of the sheets was firmly fixed on the cellulose fibrils and the leakage of the cells from the polymer matrix was not observed on the repeated batch enzyme reactions. The immobilization of cells on the surface of solid such as cellulose fibrils seemed to be possible by only radiation polymerization at low temperatures.

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