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Studies of Polystyrene-Based Ion-Exchange Fiber. V. Immobilization of Microorganism Cells by Adsorption on a Novel Fiber-Form Anion Exchanger†

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Immobilization of microorganism cells by adsorption on a new polystyrene-based ion-exchange fiber has been studied. Microorganism cells, such as yeasts, bacteria, and actinomycetes, were well adsorbed on the anion-exchange fibers through an electrostatic force. The adsorption capacity for the cells became much greater as the water-holding capacity of the fibers increased. The adsorption and desorption behavior of the cells was different between actinomycetes and yeasts, and also between strong and weak anion-exchange fibers. The enzyme activities of the immobilized actinomycetes containing glucose isomerase and yeasts containing L-aminolactam hydrolase were ca. 70 and 60% of those of the native cells respectively. The stability of the immobilized yeasts in the hydrolysis of DL-cyclic lysine anhydride was also investigated.

Recently immobilization of microorganism cells as well as enzymes has been intensively studied from a view point of their use as bioreactors in industry. Microorganism cells have been immobilized by adsorption on a support and entrapment in a matrix.[1-3]

In the previous paper,[4] we have reported that the polystyrene-based ion-exchange fiber, IONEX, can be used as a support for immobilization of some enzymes such as invertase and glucose isomerase. The ion-exchange fiber exhibits good capacity for adsorbing macromolecular ionic substances; this is attributable to its large surface area.[5-7] These findings led us to investigate the possible use of the ion-exchange fiber as the support for the immobilization of microorganism cells.[8] In the present paper, immobilization of some microorganism cells including yeasts and bacteria by adsorption on IONEX is described.

Experimental

Polystyrene for ion exchange as the predominant *sea* ingredient and polypropylene for reinforcement as the *island* ingredient were spun into composite filaments having an *islands-in-a-sea* type sectional structure shown in Fig. 1, by the conventional method.[5] Following the introduction of a crosslinking group, IONEX with various ion-exchange groups were prepared by the introduction of the corresponding ion-exchange group into the polystyrene part according to the procedure described in the previous paper.[5] The resulting fibers had a diameter of ca. 30 μm in the dry state. The ion-exchange capacities of the fibers were found to be ca. 90% of the theoretically calculated values. A cation IONEX in the Na form or an anion IONEX in the Cl form was immersed in a bath of deionized water at room temperature for a sufficient period of time to reach saturation. The fiber was centrifugally dehydrated at 3000 r.p.m. for five minutes. Immediately thereafter, the wet weight (W) of the fiber was measured. The water content of the fiber was determined from the following equation:

$$\text{Water content} = (W - W_0)/W_0,$$

where W_0 is the weight of the completely dry fiber. The water content means the water-holding capacity of the fiber and it was controlled by chosing the conditions of the crosslinking reaction.[5-7] The water content becomes higher as the degree of crosslinkage is lowered.

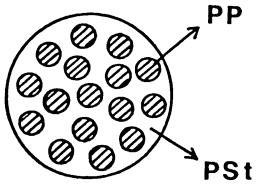

Fig. 1. Schematic cross section of composite fiber.
PSt: polystyrene, *sea* ingredient; PP: polypropylene, *island* ingredient.

Actinomycetes with the activity of glucose isomerase (G-actinomycetes; *Streptomyces phaeochromogenus*) and yeasts (P-yeasts; *Saccharomyces cerevisiae*) were obtained from Nagase Sangyo Co., Ltd. and Nitushin Seifun Co., Ltd. (Japan) respectively. Yeasts with the activity of L-aminolactam hydrolase (H-yeasts; *Cryptoccus laurentii*), bacteria (R-bacteria; *Achromobacterobae*), and bacteria (E-bacteria; *Escherichica coli*) were kindly supplied from Basic Research Laboratories, Toray Ind., Inc. (Japan).

The adsorption of the microorganism cells was carried out by the following procedure. An ion exchanger of 100 mg in the Cl or Na form was added to 0.02—0.05% cell suspension, and the mixture was shaken at 20 °C for 1 h. The adsorption capacity of the microorganism cells to the ion exchanger was evaluated by measuring the concentration of the cells remaining in the suspension before and after the adsorption treatment by optical-density measurements at 550 nm. Glucose isomerase activity was determined as described in the previous paper.[4] One unit (U) of L-aminolactam hydrolase was defined as the amount of the enzyme which produced 1 μ mole of L-lysine per hour at 40 °C in 10 wt/vol% DL-cyclic lysine anhydride (CLA) solution of pH 8.0 containing 0.625 mM $MnCl_2$ (1 M=1

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mol dm^{-3}).

Results and Discussion

Adsorption of Microorganism Cells. The first experiment was carried out using G-actinomycetes, H-yeasts, R-bacteria, and P-yeasts as the test microorganism cells to test whether microorganism cells were adsorbed on IONEX. The results are summarized in Table 1. E-Bacteria of 25 mg and 4 mg were also adsorbed from 0.115% cell suspension (20 °C, 4 h) on 100 mg of anion IONEX having a dimethylaminomethyl group with a water content of 7.0 and Amberlite IRA-938 respectively. It can be seen from Table 1 that the microorganism cells are not adsorbed on cation IONEX with a sulfo group, but well adsorbed on anion IONEX. They are slightly adsorbed on the macroreticular-type anion-exchange resin (Amberlite IRA-938). These results suggest that the outer walls of the microorganism cells are negatively charged and are adsorbed on anion exchangers by an electrostatic force.

Figure 2 shows the relation between the cell adsorption capacity and the water content of anion IONEX. The adsorption capacity was dependent on the water content of the fiber, but independent of the kind of anion-exchange group. The adsorption capacity of the actinomycetes and bacteria was lower than that of the yeasts. This can be ascribed to the difference in the cell size. The strong anion IONEX with water contents of 1.0 and 4.0 had adsorption capacities of 60 and 700 mg g^{-1} for P-yeasts respectively. The adsorption capacity of the yeasts on the fiber can be geometrically evaluated from their volumes and surface areas. We assume, as a first-order approximation, that the spherical yeasts with a diameter of 6 μm are most closely adsorbed on the surface of the cylindrical endless fiber with a variable

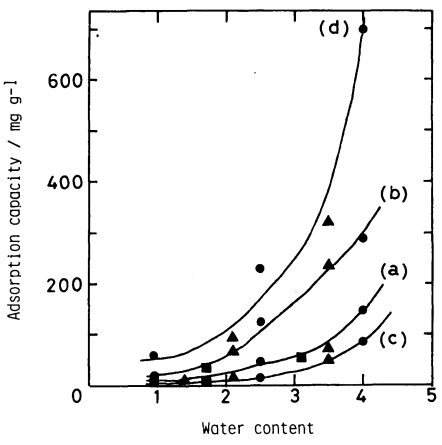

Fig. 2. Cell adsorption capacity vs. water content of anion IONEX. (a) G-Actinomycetes, (b) H-yeasts, (c) R-bacteria, (d) P-yeasts. ●: Trimethylammonio group, ■: dimethylamino group, ▲: amino group.

Table 1. Results of Microorganism Cell Adsorption on Various Exchangers

Exchanger[a]	Ionic group	Water content	G-Actinomycetes		H-Yeasts		R-Bacteria		P-Yeasts	
			Added[b]	Adsorbed[e]	Added[c]	Adsorbed[e]	Added[c]	Adsorbed[e]	Added[d]	Adsorbed[e]
			mg	mg	mg	mg	mg	mg	mg	mg
Strong anion IONEX	-φ-$CH_2\overset{+}{N}(CH_3)_3$	1.0	4	1.4	10	3.1	10	0.5	20	6.0
		2.5	12	4.8	10	9.1	10	1.8	50	23
		4.0	40	14.5	50	29.3	10	8.5	200	70
Weak anion IONEX	-φ-$CH_2\overset{+}{N}H(CH_3)_2$	3.1	8	5.3	—	—	—	—	—	—
	-φ-$CH_2\overset{+}{N}H_3$	3.5	10	7.2	30	23.4	10	8.4	100	32
Strong cation IONEX	-φ-SO_3^-	3.1	4	0.0	10	0.0	10	0.0	10	0.0
Amberlite IRA-938	-φ-$CH_2\overset{+}{N}(CH_3)_3$	2.7	—	—	10	1.7	10	0.5	10	2.1

a) 100 mg. b) 0.02% Suspension (0.05 M $NaHCO_3$, pH 8.2), (distilled water) for weak anion IONEX. c) 0.05% Suspension (0.05 M Tris buffer, pH 8.0), (distilled water) for weak anion IONEX. d) 0.05% Suspension (distilled water). e) 20 °C, 1 h.

diameter according to the water content (30 μm in dry state). The adsorption capacities for the fibers with water contents of 1.0 and 4.0 are calculated to be 680 and 1020 mg g^{-1} respectively. In the case of the yeasts, the experimental values of the adsorption capacity are generally lower than the calculated ones. This discrepancy is explained by assuming that the adsorption capacity is reduced by the repulsion between the cells, rather than that the adsorption capacity is not saturated because of the low concentration of the cells. On increasing the water content of the fiber, the adsorption capacity is considered to increase rapidly and to approach the calculated capacity since the adherent force of the cells to the fiber is enhanced by their affinity for water and it inhibits the repulsion between the cells.

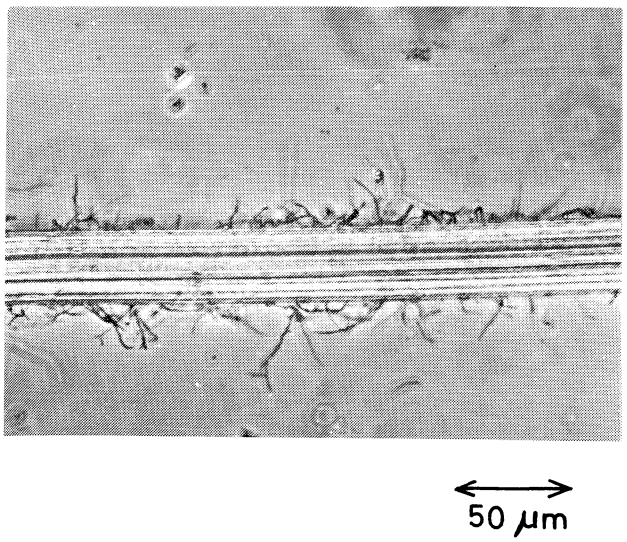

Fig. 3. Adsorption process of G-actinomycetes on strong anion IONEX in 0.05 M $NaHCO_3$ solution (pH 8.2).

Adsorption and Desorption Behavior of Microorganism Cells. Figure 3 presents the adsorption process of G-actinomycetes on the strong anion IONEX. The extreme part of the cells adheres on the fiber surface at first when the fiber is added to the cell suspension. The whole of the cell adheres on the fiber surface on shaking.

Figures 4 and 5 show G-actinomycetes and H-yeasts adsorbed on the strong anion IONEX. The yeasts were easily desorbed from the strong anion IONEX in a 10 wt% NaCl or lysine aqueous solution (Fig. 6). However, vigorous shaking was needed for desorption of G-actinomycetes even in 10 wt% NaCl. The results suggest that the ionic strength in a solution controls the adsorption and desorption properties for the cells in the case of a strong anion exchanger. The fiber is considered to shrink and lose the electrostatic force in a solution with a high ionic strength. The reason why this change of the

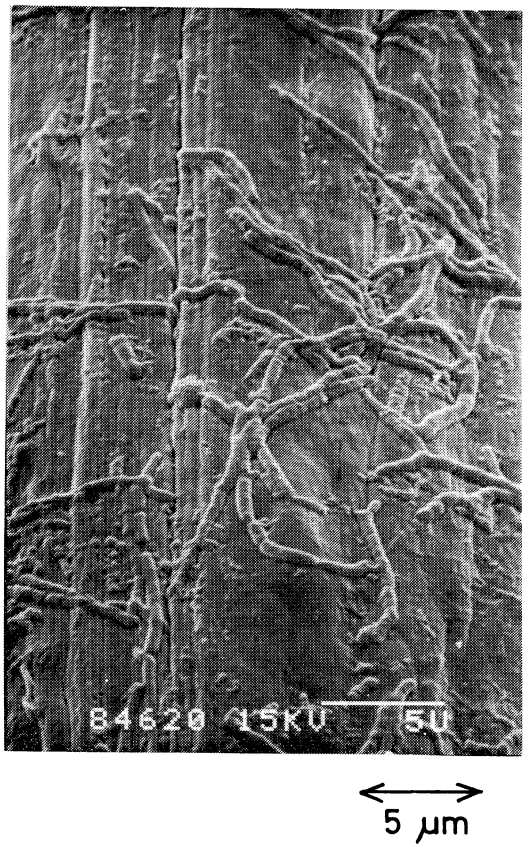

Fig. 4. Adsorption state of G-actinomycetes on strong anion IONEX of water content, 2.5.

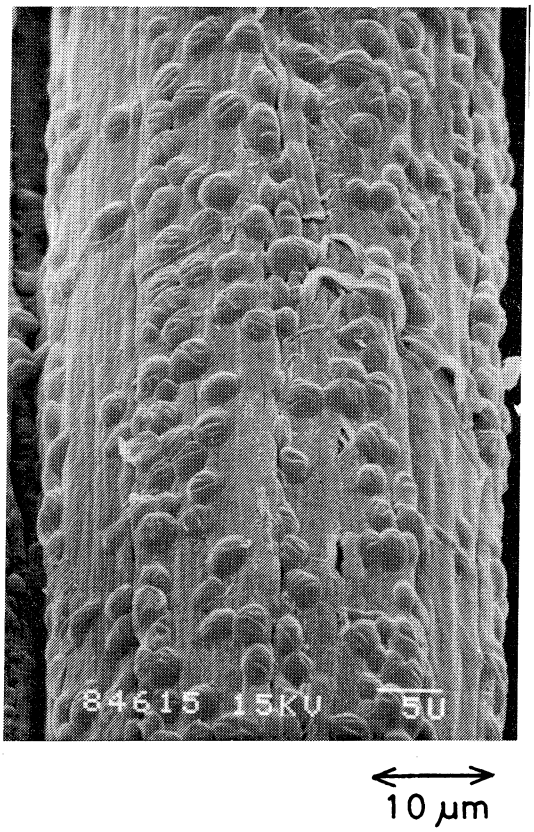

Fig. 5. Adsorption state of H-yeasts on strong anion IONEX of water content, 2.5.

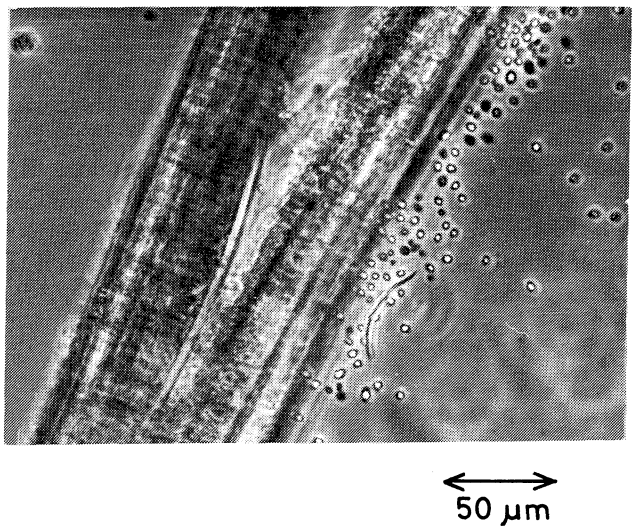

Fig. 6. Desorption process of H-yeasts from strong anion IONEX in 10 wt% NaCl solution.

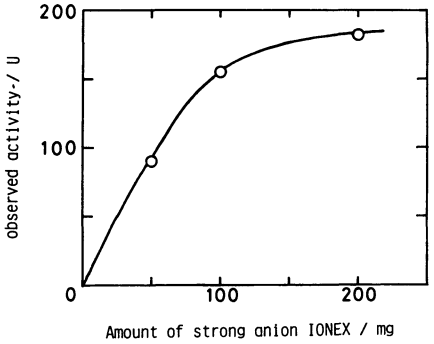

Fig. 7. Observed activity of glucose isomerase vs. amount of strong anion IONEX (water content, 4.0). Immobilization was carried out in 25 ml of 0.05 M $NaHCO_3$ (pH 8.2) containing G-actinomycetes of 260 U, 50 mg (20 °C, 1 h).

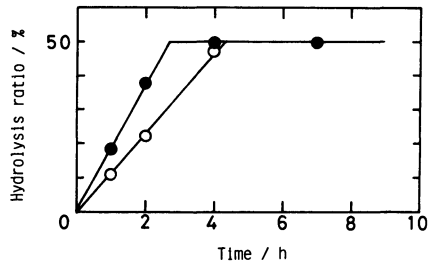

Fig. 8. Hydrolysis ratio of DL-CLA as a function of time. Native or immobilized cells were placed in 10 ml of 10 wt/vol% DL-CLA solution (pH 8.0) containing 0.625 mM $MnCl_2$ and shaken at 40 °C. ●: Native H-yeasts (17.5 mg), 1455 U, ○: immobilized H-yeasts (17.5 mg) on 500 mg of weak anion IONEX with amino group, 845 U.

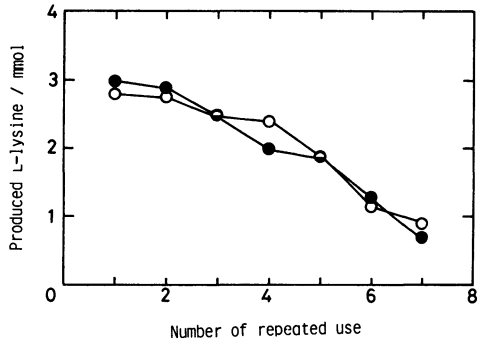

Fig. 9. Stability of immobilized H-yeasts in hydrolysis of DL-CLA by a repeated batch method. Immobilized H-yeasts (1.75 mg) on 25 mg of weak anion IONEX with amino group were placed in 8 ml of 10 wt/vol% DL-CLA solution (pH 8.0) containing 0.625 mM $MnCl_2$ and shaken at 40 °C for 40 h. 0.7 mg of native H-yeasts produced 2.45 mmol of L-lysine on the same conditions. The open and closed circles represent two separate experimental data.

electrostatic force does not readily cause the desorption of the actinomycetes can be attributed to the difference in the adsorption state. That is, the actinomycetes have a much larger contact area with the fiber than the yeasts, as seen in Figs. 4 and 5, so that the former is not so easily desorbed from the fiber as the latter. In the case of the weak anion IONEX, no appreciable desorption of the yeasts was observed in a 10 wt% lysine aqueous solution. They were partially desorbed from the fiber in a 10 wt% NaCl aqueous solution and extensively in a 1 M NaOH. Therefore, the adsorption and desorption properties of the cells for a weak anion exchanger are affected by the pH of a solution rather than by the ionic strength. This is considered to be due to the fiber shrinking more as the pH in a solution is raised. The adsorption and desorption properties for R-bacteria were the same as for H-yeasts.

Immobilization of Microorganism Cells.

Figure 7 shows the correlation between the amount of the strong anion IONEX and the glucose isomerase activity of the fiber when a fixed amount of G-actinomycetes is used in the immobilization. The activity yield was relatively high at ca. 70%.

The hydrolysis of DL-CLA was carried out by using the native H-yeasts with the activity of L-aminolactam hydrolase and the immobilized H-yeasts on the weak anion IONEX with the amino group, as is shown in Fig. 8. The activity yield was ca. 60%. Next, the stability of the immobilized H-yeasts in the hydrolysis of DL-CLA was investigated by a repeated batch method (40 °C, 40 h). The plots of the L-lysine produced vs. the number of repeated use are given in Fig. 9. It can be seen from Fig. 9 that the half life of the immobilized H-yeasts is ca. 240 h. The decrease in activity can be attributed to the inactivation of the enzyme since no appreciable desorption of the yeasts was observed during the reaction. The weak anion IONEX for the support can be repeatedly used by immobilizing new H-yeasts after the desorption

treatment in a 1 M NaOH aqueous solution when the activity of the immobilized H-yeasts decreases.

Conclusion

1. The microorganism cells are well adsorbed on anion-exchange fiber. On increasing the water content of the fiber as well as its surface area, the adsorption ability for the immobilization becomes much larger.
2. The adsorption and desorption behavior is dependent upon the kind of microorganism cells and also upon the basicity of the anion-exchange fiber.
3. The immobilization of the microorganism cells by adsorption on the fiber gives a high enzyme activity.

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