Skin-Layer Formation on Porous Membrane by Immobilized Dextransucrase

Hidetaka Kawakita, Kyoichi Saito, and Kazuyuki Sugita
Dept. of Materials Technology, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan

Masao Tamada and Takanobu Sugo
Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma 370-1292, Japan

Hiroshi Kawamoto
Kikkoman Corp., 399 Noda, Noda, Chiba 278-0037, Japan

A porous hollow-fiber membrane with an average pore diameter of 360 nm and a porosity of 71% was used as a starting polymer. An epoxy-group-containing monomer, glycidyl methacrylate, was grafted onto the porous hollow-fiber membrane by radiation-induced graft polymerization. The produced epoxy group was quantitatively converted into a 2-hydroxyethylamino group as an anion-exchange group by reaction with ethanolamine. Dextransucrase solution was forced to permeate radially outward through the pore from the inside surface of the porous hollow-fiber membrane to be immobilized by the grafted polymer chains based on an anion-exchange interaction. Subsequently, sucrose solution as substrate was fed to the inside surface of the dextransucrase-immobilized porous hollow-fiber membrane. Dextran produced by the enzymatic reaction formed a skin layer on the inside surface of the membrane with a thickness of approximately 4 μm and an estimated pore diameter of 50 nm. © 2004 American Institute of Chemical Engineers
AIChE J, 50: 696–700, 2004
Keywords: porous hollow-fiber membrane, radiation-induced graft polymerization, immobilized dextran sucrase, dextran, skin layer

Introduction

Dextransucrase functions as a catalyst to produce dextran and fructose from sucrose (Kobayashi and Matsuda, 1974, 1980; Girard and Legoy, 1999). Dextran as a product forms a complex with an active site of dextransucrase (Robyt et al., 1974; Robyt and Corrigan, 1977; Mayer et al., 1981; Mooser and Iwaoka, 1989). Progression of the enzymatic reaction allows dextran to increase its molecular mass; the overall activity of dextransucrase immobilized to a beam gradually decreases, because dextran with increasing molecular mass restricts the diffusion of sucrose (Kaboli and Reilly, 1980; Chang et al., 1981; Monsan et al., 1981; 1987).

We have, in the past, prepared ion-exchange porous hollow-fiber membranes for the immobilization of enzymes by radiation-induced graft polymerization and subsequent chemical modifications. Aminoacylase (Nakamura et al., 1998; Kawai et al., 2001a), ascorbic acid oxidase (Kawai et al., 2001b), and cycloisomaltooligosaccharide glucanotransferase (Kawai et al., 2002; Kawakita et al., 2002a,b) were immobilized by ion-exchange polymer chains grafted onto the pore surfaces of porous hollow-fiber membranes. A substrate solution was forced to permeate through the pore driven by a transmembrane pressure, resulting in the minimization of the diffusional path

Correspondence concerning this article should be addressed to K. Saito at marukyo@xtal.tf.chiba-u.ac.jp.

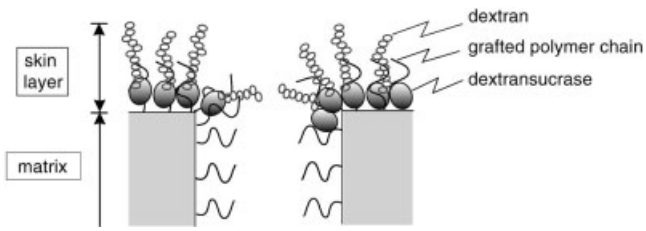

Figure 1. Principle of skin-layer formation by dextran produced by enzymatic reaction.

of the substrate to the enzyme immobilized by the polymer chains.

In this study, we suggest a novel method for generating a skin layer on a porous hollow-fiber membrane by feeding sucrose as a substrate to dextransucrase-immobilized polymer chains grafted onto the pore surface of the porous hollow-fiber membrane. Dextran produced by the enzymatic reaction formed a skin layer on the inside surface of the porous hollow-fiber membrane, as illustrated in Figure 1. The pore size of the skin layer was estimated from the liquid permeability of the porous hollow-fiber membrane and the thickness of the skin layer generated on the inside surface.

The conventional techniques for generating a skin layer on porous membranes include surface polymerization (Kim et al., 2002; Roh, 2002), dynamic method (Ohtani et al., 1991; Tsapiuk, 1996), and graft polymerization (Yamaguchi et al., 1991, 1992; Kai et al., 2000). The technique presented in this study has two features: (1) the structure of the skin layer is controllable by various parameters for binding DSase and subsequent enzymatic reactions, and (2) the dextran-made skin layer renders the membrane surface hydrophilic to reduce fouling of the surface.

Materials and Methods

Materials

A porous hollow-fiber membrane, made of polyethylene, was supplied by Asahi Kasei Corporation and used as a starting polymer for grafting. This hollow fiber had inner and outer diameters of 1.9 and 3.1 mm, respectively, with an average pore size of 0.36 μm and a porosity of 71%. Dextransucrase was purchased from Sigma (D-9909, Lot No. 18H4075). Glycidyl methacrylate (GMA, $CH_2C{=}CCH_3COOCH_2CHOCH_2$) was purchased from Tokyo Kasei Co. and used without further purification. Other reagents were of analytical grade or higher.

Preparation of anion-exchange porous hollow-fiber membrane

The preparation scheme of an anion-exchange porous hollow-fiber membrane for enzyme immobilization is shown in Figure 2. The procedures were detailed in our previous publication (Tsuneda et al., 1995). Briefly, the porous hollow-fiber membrane as a starting polymer was irradiated with a cascade-type accelerator in nitrogen atmosphere at ambient temperature. The dose was 200 kGy. The irradiated starting polymer was immersed in 10 (v/v)% GMA/methanol solution at 313 K. The degree of GMA grafting, defined by the following, was set at 160% by adopting a reaction time of 12 min:

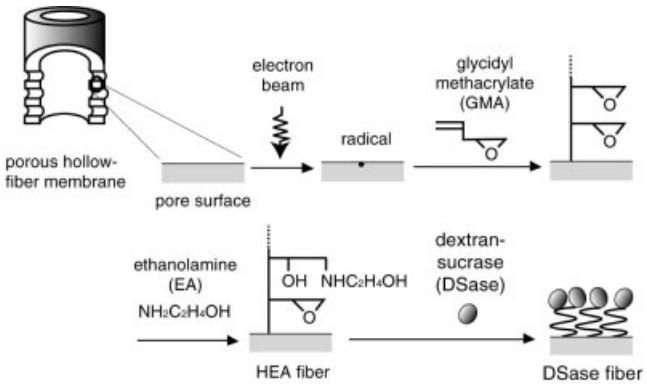

Figure 2. Preparation scheme of dextransucrase-immobilized porous hollow-fiber membrane.

$$\text{Degree of GMA grafting} = 100\ (\text{mass of the poly-GMA chains grafted})/(\text{mass of the starting polymer}) \quad (1)$$

Subsequently, in order to convert the epoxy group of the grafted polymer chains into a 2-hydroxyethylamino (HEA) group as an anion-exchange group, the GMA-grafted porous hollow-fiber membrane was immersed in ethanolamine at 303 K for 24 h. The molar conversion of the epoxy group into the HEA group was calculated from the weight gain. The resultant porous hollow-fiber membrane was referred to as an HEA fiber.

Binding of dextransucrase to anion-exchange porous hollow-fiber membrane

The HEA fiber with an effective length of 2 cm was positioned as shown in Figure 3. Two U/mL dextransucrase buffered with 0.1 M acetate buffer (pH 5.5) was forced to permeate through the pore of the HEA fiber using a syringe pump (ATOM, 1235N) at a constant permeation rate of 10 mL/h at 296 K. The effluent penetrating the outside surface of the HEA fiber was continuously collected and the activity of dextransucrase in the effluent was determined. The activity of 1 U was defined as the amount of enzyme required to produce 1 μm fructose for one minute. When a dimensionless effluent volume (DEV), defined as the ratio of the effluent volume to the membrane volume including the lumen part, reached ten, the adsorption procedure was switched to the washing procedure

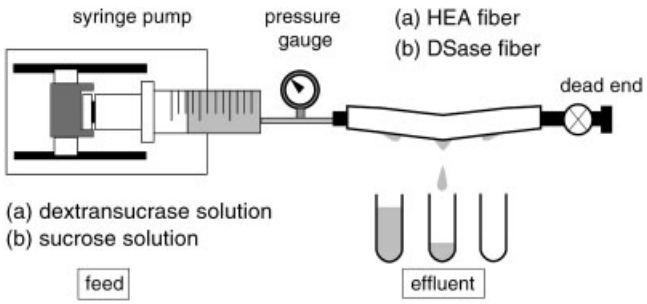

Figure 3. Experimental apparatus for enzyme immobilization and substrate permeation.

Table 1. Properties of Porous Hollow-Fiber Membranes

	HEA Fiber	Starting Fiber
Size (mm)		
Inner diameter	2.6	1.9
Outer diameter	4.1	3.1
Porosity (%)	74	71
Specific surface area (m^2/g)	5.5	14
Pure water flux (m/h)*	1.6	2.5
Functional group density (mmol/g)	2.1	—

* Transmembrane pressure: 0.1 MPa; temperature: 298 K.

with acetate buffer. The amount of dextransucrase bound to the HEA fiber was evaluated from the following integration:

Amount of dextransucrase bound to the HEA fiber

$$= \int_0^V (C_0 - C)dV/W \quad (2)$$

where C_0 and C are the dextransucrase concentrations of the feed and the effluent, respectively, and V and W are the effluent volume and the mass of the HEA fiber, respectively. The resultant porous hollow-fiber membrane was referred to as a DSase(x) fiber, where x designates the amount of bound dextransucrase.

Production of dextran during permeation of sucrose solution

Four g/L sucrose buffered with the acetate buffer was permeated radially outward from the inside surface of the DSase(60) fiber to the outside surface at a constant permeation rate of 10 mL/h at 296 K until a DEV of the sucrose solution of 8.2 is reached. This enzymatic reaction is expressed by

$$G_{N-1} + \text{sucrose} \rightarrow G_N + \text{fructose} \quad (3)$$

where G and the subscript N designate dextran and the degree of polymerization of glucose, respectively. Fructose was determined by high-performance liquid chromatography using an Amide-80 column (Tosoh). The amount of dextran produced per inside surface area of the HEA fiber was evaluated as

Amount of dextran produced per inside surface area

$$= \int_0^V C_F dV/(\text{inside surface area}) \quad (4)$$

where C_F is the fructose concentration in the effluent. The resultant porous hollow-fiber membrane was referred to as a Dex(x, y) fiber, where x and y are the amount of bound dextransucrase and the amount of dextran produced per inside surface area of the DSase(x) fiber, respectively.

The permeation pressure required for realizing a constant permeation rate of pure water through the pore of the Dex(60, y) fiber was determined at 296 K. After the Dex(60, 27) fiber was dried under reduced pressure, the inside surface of the fiber was observed by scanning electron microscopy (SEM) to measure the thickness of the skin layer consisting of dextran produced by the enzymatic reaction.

Results and Discussion

Immobilization of dextransucrase onto anion-exchange porous hollow-fiber membrane

The properties of the anion-exchange porous hollow-fiber membrane are summarized in Table 1. The molar conversion of the epoxy group into the 2-hydroxyethylamino (HEA) group for a reaction time of 24 h amounted to 90%. The HEA fiber swelled in thickness by 25% compared with the starting porous hollow-fiber membrane. The introduction of the HEA group into the graft chain embedded in the polyethylene matrix by graft polymerization of GMA induced the swelling (Tsuneda et al., 1992). The pure water flux, defined by dividing the permeation rate of pure water by the inside surface area of the hollow fiber, of the HEA fiber decreased to 64% of that of the starting porous hollow-fiber membrane.

The amount of dextransucrase bound to the HEA fiber as a function of DEV of dextransucrase solution is shown in Figure 4. The amount of bound dextransucrase linearly increased with increasing DEV and amounted to 60 U per g of the HEA fiber for a DEV of the dextransucrase solution of 8.3.

Skin-layer formation on porous hollow-fiber membrane by dextran production

The amount of dextran produced by feeding sucrose solution to the DSase(60) fiber as a function of the DEV of the sucrose solution is shown in Figure 5. The amount of dextran increased exponentially with increasing DEV of sucrose solution. No dextran was detected in the effluent penetrating the outside surface of the DSase(60) fiber; therefore, all of the dextran produced by the dextransucrase immobilized by the grafted polymer chain was found to be retained by the porous hollow-fiber membrane. For example, the amount of produced dextran

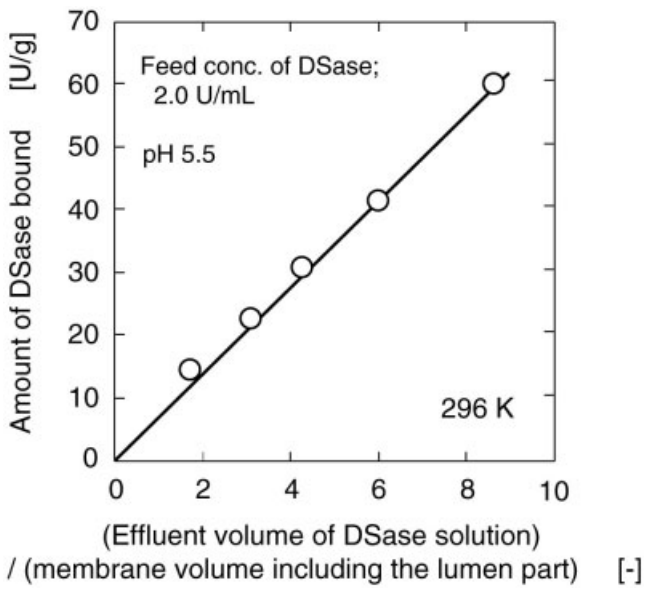

Figure 4. Amount of DSase bound to the HEA fiber.

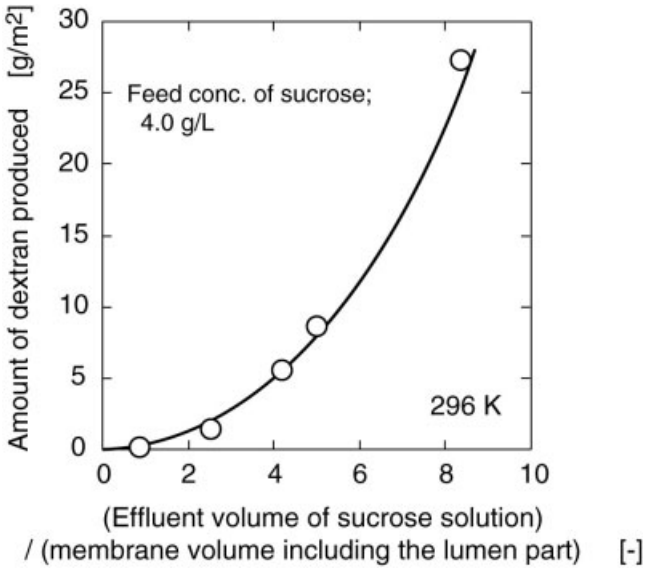

Figure 5. Amount of dextran produced during the permeation of sucrose solution through the DSase(60) fiber.

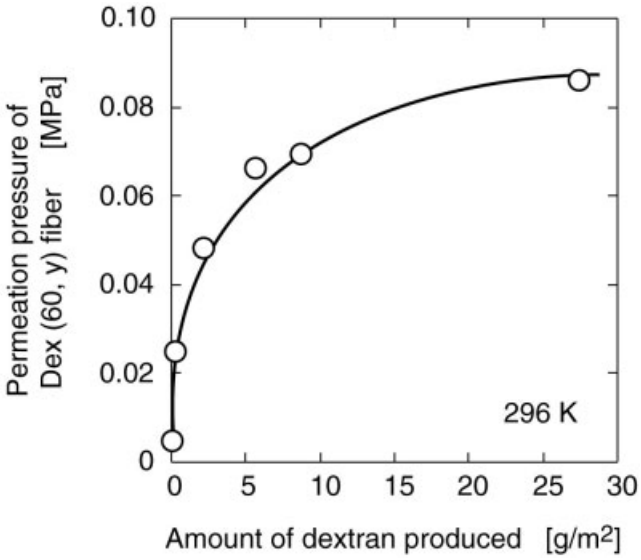

Figure 7. Permeation pressure of the Dex(60, y) fiber required for a constant permeation rate of pure water of 10 mL/h.

was calculated as 27 g/m^2 for a DEV of sucrose solution of 8.2 by Eq. 4.

A SEM image of the inside surface of the resultant Dex(60, 27) fiber is shown in Figure 6 along with that of the DSase(60) fiber. The dextran produced formed a skin layer on the inside surface of the DSase(60) fiber with a thickness of approximately 4 μm: the resultant Dex(60, 27) fiber consists of the dextran layer as a skin layer on the inside surface and the DSase fiber as a supporting matrix.

Estimation of pore size of skin layer

The permeation pressure required for a constant permeation rate of pure water of 10 mL/h through the Dex(60, y) fiber as a function of y is shown in Figure 7. An increase in the amount of dextran produced resulted in an increase in the permeation pressure. For example, the permeation pressure for the Dex(60, 27) fiber was 20-fold that for the DSase(60) fiber.

The pore size of the skin layer consisting of dextran was

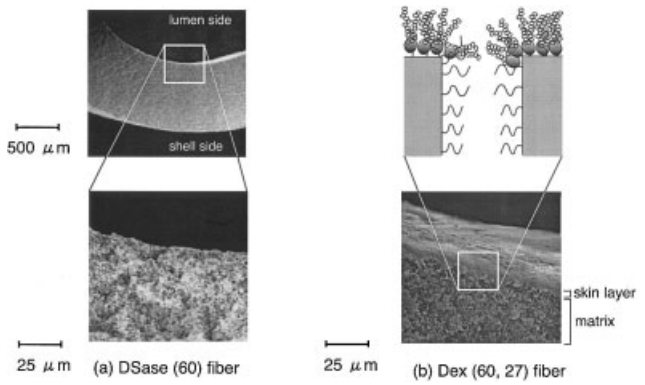

Figure 6. SEM images of inside surface of the porous hollow-fiber membrane: (a) DSase(60) fiber, and (b) Dex(60, 27) fiber.

estimated under the following three assumptions: (1) parallel straight pores penetrate the membrane uniformly; (2) the permeation pressure for the Dex(x, y) fiber is the sum of the pressure loss across the skin layer and across the matrix, that is, the DSase fiber, and the pressure loss is expressed by the Hagen-Poiseuille equation at a laminar flow regime; and (3) the pore number density of the skin layer is the same as that of the matrix.

The permeation pressure P_T is

$$P_T = P_S + P_M = 8\mu F[(L_s/(n_s\pi r_s^4)) + (L_M/(n_M\pi r_M^4))] \quad (5)$$

where P is pressure loss; subscripts S and M designate the skin layer and the matrix, respectively; L, n, and r are thickness, pore number density, and pore radius of the porous hollow-fiber membrane, respectively; and F and μ are the flux and viscosity of liquids, respectively. For the values for the matrix or the DSase(60) fiber, L_M, r_M, and n_M are 0.75 mm, 190 nm, and 5.5 $\times$ 10^{12} m^{-2}, respectively. The pore number density was calculated by applying the Hagen-Poiseuille equation to the analysis of pure water flux. The thickness of the skin layer of L_S is 4 μm from SEM observation (Figure 6), and the pore number density is assumed to be identical to n_M. The insertion of the measured value of P_T under a constant pure water flux F (Figure 7) into Eq. 5 yielded the pore radius of the skin layer of 24 nm.

Conclusion

A novel technique for generating a skin layer on the surface of a porous hollow-fiber membrane via an enzymatic reaction was suggested in this study. In order to immobilize dextransucrase onto the pore surface of the porous hollow-fiber membrane, an anion-exchange-group-containing polymer chain was appended onto the pore surface by radiation-induced graft

polymerization of glycidyl methacrylate and subsequent conversion of the epoxy group into a 2-hydroxyethylamino group. Dextransucrase was bound to the inside surface of the anion-exchange porous hollow-fiber membrane. Subsequent permeation of a sucrose solution as a substrate through the dextransucrase-immobilized porous hollow-fiber membrane generated the skin layer that consisted of the complex of the produced dextran with the active site of dextransucrase. The skin layer covered the inside surface of the porous hollow-fiber membrane with a thickness of approximately 4 μm and the estimated pore diameter of 50 nm. This technique for skin layer formation by use of an enzymatic reaction on the pore surface provides potential applications for membrane preparation with tailored pore size.

Acknowledgment

We thank Kohei Watanabe and Noboru Kubota of the Industrial Membranes Division of Specialty Products & Systems Company of Asahi Kasei Corporation for providing the starting porous hollow-fiber membrane.

Notation

C = dextransucrase concentration in the effluent, U/mL
C_0 = dextransucrase concentration in the feed, U/mL
C_F = fructose concentration in the effluent, g/L
DEV = dimensionless effluent volume
Dex fiber = porous hollow-fiber membrane containing skin layer that consists of dextran produced by permeating a sucrose solution through DSase fiber
DSase fiber = dextransucrase-immobilized porous hollow-fiber membrane
HEA fiber = porous hollow-fiber membrane containing a 2-hydroxyethylamino group
F = flux of porous hollow-fiber membrane, m/h
G = dextran
GMA = glycidyl methacrylate
L = thickness of the porous hollow-fiber membrane, μm
N = degree of polymerization of glucose
n = pore number density of the porous hollow-fiber membrane, m^{-2}
P = pressure loss, MPa
r = pore radius of the porous hollow-fiber membrane, m
V = effluent volume, mL
W = mass of the HEA fiber, g
x = amount of bound dextransucrase, U/g
y = amount of dextran produced per inside surface area, g/m^2
μ = viscosity, Pa · s

Literature Cited

Chang, H. N., Y. S. Chim, Y. R. Cho, D. A. Landis, and P. J. Reilly, "Immobilization of *Leuconostoc mesenteroides* Dextransucrase to Porous Phenoxyacetyl Cellulose Beads," *Biotechnol. Bioeng.,* **23,** 2647 (1981).

Girard, E., and M.-D. Legoy, "Activity and Stability of Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F in the Presence of Organic Solvents," *Enzyme Microb. Technol.,* **24,** 425 (1999).

Kaboli, H., and P. J. Reilly, "Immobilization and Properties of *Leuconostoc mesenteroides* Dextransucrase," *Biotechnol. Bioeng.,* **22,** 1055 (1980).

Kai, T., T. Yamaguchi, and S. Nakao, "Preparation of Organic/Inorganic Composite Membranes by Plasma-Graft Filling Polymerization Technique for Organic-Liquid Separation," *Ind. Eng. Chem. Res.,* **39,** 3284 (2000).

Kawai, T., M. Nakamura, K. Sugita, K. Saito, and T. Sugo, "High-Conversion in Asymmetric Hydrolysis during Permeation Through Enzyme-Multilayered Porous Hollow-Fiber Membrane," *Biotechnol. Prog.,* **17,** 872 (2001a).

Kawai, T., K. Saito, K. Sugita, T. Sugo, and H. Misaki, "Immobilization of Ascorbic Acid Oxidase in Multilayers onto Porous Hollow-Fiber Membrane," *J. Membr. Sci.,* **191,** 207 (2001b).

Kawai, T., H. Kawakita, K. Sugita, K. Saito, M. Tamada, T. Sugo, and H. Kawamoto, "Conversion and Dextran to Cycloisomaltooligosaccharides Using an Enzyme-Immobilized Porous Hollow-Fiber Membrane," *J. Agric. Food Chem.,* **50,** 1073 (2002).

Kawakita, H., K. Sugita, K. Saito, M. Tamada, T. Sugo, and H. Kawamoto, "Production of Cycloisomaltooligosaccharides from Dextran Using Enzyme Immobilized in Multilayers onto Porous Membranes," *Biotechnol. Prog.,* **18,** 465 (2002a).

Kawakita, H., K. Sugita, K. Saito, M. Tamada, T. Sugo, and H. Kawamoto, "Optimization of Reaction Conditions in Production of Cycloisomaltooligosaccharides Using Enzyme Immobilized in Multilayers onto Pore Surface of Porous Hollow-Fiber Membranes," *J. Membr. Sci.,* **205,** 175 (2002b).

Kim, I.-C., H.-G. Yun, and K.-H. Lee, "Preparation of Asymmetric Polyacrylonitrile Membrane with Small Pore Size by Phase Inversion and Post-Treatment Process," *J. Membr. Sci.,* **199,** 75 (2002).

Kobayashi, M., and K. Matsuda, "The Dextransucrase Isoenzymes of *Leuconostoc mesenteroides* NRRL B-1229," *Biochim. Biophys. Acta,* **370,** 441 (1974).

Kobayashi, M., and K. Matsuda, "Characterization of the Multiple Forms and Main Component of Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F," *Biochim. Biophys. Acta,* **614,** 46 (1980).

Mayer, R. M., M. M. Matthews, C. L. Futerman, V. K. Parnaik, and S. M. Jung, "Dextransucrase: Acceptor Substrate Reactions," *Arch. Biochem. Biophys.,* **208,** 278 (1981).

Monsan, P., and A. Lopez, "On the Production of Dextran by Free and Immobilized Dextransucrase," *Biotechnol. Bioeng.,* **23,** 2027 (1981).

Monsan, P., F. Paul, D. Auriol, and A. Lopez, "Dextran Synthesis Using Immobilized *Leuconostoc mesenteroides* Dextransucrase," *Methods Enzymol.,* **136,** 239 (1987).

Mooser, G., and K. R. Iwaoka, "Sucrose 6-α-D-Glucosyltransferase from *Streptococcus sobrinus:* Characterization of a Glucosyl-Enzyme Complex," *Biochemistry,* **28,** 443 (1989).

Nakamura, M., K. Saito, K. Sugita, and T. Sugo, "Application of Crosslinked-Aminoacylase-Multilayered Membranes to Bioreactor," *Maku* (*Membrane*) **23,** 316 (1998).

Ohtani, T., M. Nakajima, Y. Nawa, and A. Watanabe, "Formation of Dynamic UF Membrane with Fine Zr Particles," *J. Membr. Sci.,* **64,** 273 (1991).

Robyt, J. F., B. K. Kimble, and T. F. Walseth, "The Mechanism of Dextransucrase Action, Direction of Dextran Biosynthesis," *Arch. Biochem. Biophys.,* **165,** 634 (1974).

Robyt, J. F., and A. J. Corrigan, "The Mechanism of Dextransucrase Action, Activation of Dextransucrase from *Streptococcus mutans* OMZ 176 by Dextran and Modified Dextran and the Nonexistence of the Primer Requirement for the Synthesis of Dextran," *Arch. Biochem. Biophys.,* **183,** 726 (1977).

Roh, I. J., "Influence of Rupture Strength of Interfacially Polymerized Thin-Film Structure on the Performance of Polyamide Composite Membranes," *J. Membr. Sci.,* **198,** 63 (2002).

Tsapiuk, E. A., "Ultrafiltration Separation of Aqueous Solutions of Poly(Ethylene Glycol)s on the Dynamic Membrane Formed by Gelatin," *J. Membr. Sci.,* **116,** 17 (1996).

Tsuneda, S., K. Saito, S. Furusaki, T. Sugo, and I. Ishigaki, "Water/Acetone Permeability of Porous Hollow-Fiber Membrane Containing Diethylamino Groups on the Grafted Polymer Branches," *J. Membr. Sci.,* **71,** 1 (1992).

Tsuneda, S., H. Kagawa, K. Saito, and T. Sugo, "Protein Adsorption Characteristics of Porous and Tentacle Anion-Exchange Membrane Prepared by Radiation-Induced Graft Polymerization," *Radiat. Phys. Chem.,* **46,** 239 (1995).

Yamaguchi, T., S. Nakao, and S. Kimura, "Plasma-Graft Filling Polymerization: Preparation of a New Type of Pervaporation Membrane for Organic Liquid Mixtures," *Macromolecules,* **24,** 5522 (1991).

Yamaguchi, T., S. Nakao, and S. Kimura, "Solubility and Pervaporation Properties of the Filling-Polymerized Membrane Prepared by Plasma-Graft Polymerization for Pervaporation of Organic-Liquid Mixtures," *Ind. Eng. Chem. Res.,* **31,** 1914 (1992).

Manuscript received Dec. 4, 2002, and revision received June 10, 2003.