Biotechnology and Bioprocess Engineering **2004**, 9: 500-505

Purification During Crossflow Electromicrofiltration of Fermentation Broth

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Abstract The present study was to investigate the purification of a fermentation broth by an electromicrofiltration membrane. Microfiltration runs with a crude and a centrifuged broth, with solution of particles recovered from centrifugation and with permeates from microfiltration experiments were thus compared. Microfiltration performances were governed by colloids and small particles that induced sharp initial flux declines. For these results, the evolution of the overall membrane resistance was increased by 80% in comparison with the electromicrofiltration membrane. The main focus of this study was set on the enhancement of the filtrate flux by an electric field. This pressure electrofiltration leads to a drastic improvement of the filtration by 100% and the filtration time was thereby reduced. Pressure electrofiltration serves as an interesting alternative to the cross-flow filtration and it effectively separates advantageous constituents such as amino acids and biopolymers from a fermentation broth. They were equally maintained during the microelectrofiltration, although they were significantly reduced by 45% by the microfiltration without the application of an electric field. Accordingly, since the electrofiltration membrane was provided more permeability, this study experimentally demonstrates that the permeability inside a membrane can be controlled using an electric field.

Keywords: fermentation, microfiltration, broth, centrifugation, electric field, electrofiltration

INTRODUCTION

Fermented broth conventionally undergoes two processing steps. Crude broth following alcoholic fermentation includes numerous solutes such as organic acids, salts, macromolecules and colloidal size-range aggregates and particles, microorganisms and various large particles such as cell debris. First, it is filtered to remove polysaccharides and dissolved constituents, colloids and large particles, resulting in a filtered broth. This is usually achieved by filtration with a filter aid or by centrifugation. Second, the use of membranes with a pore size of 0.45 μm and below may potentially purify the broth. The cross-flow microfiltration development has long been hampered by significant fouling of the membrane despite definite advantages of membrane purification. The consequence of this is a reduction in permeation rates, affecting the economic viability of the process, and a risk of excessive polysaccharide retention, likely to affect product organoleptic quality. Fouling depends highly not only on the broth being processed, but also on the membrane and process condition. Advances in materials combined with the introduction of backflow have already allowed to significantly reduce retention problems, allowing the implantation of the technique [1]. The major problem is facility variability that makes purification-facility dimensioning difficult according to the fermentation broth. The irreversibility of fouling, ascribed to physico-chemical interactions between components present in fermented broth and membranes, results in cleaning difficulties. The fouling is formed during microfiltration, thus it is of crucial importance to manage the following points: (1) to be in position to control or reduce it; (2) to improve cleaning methods; (3) and to newly adapt the material and/or the process to the product to be filtered.

The present study performed the filtration experiments with a focus on the beverage industry, for example, the purification of a biopolymer and the recovery of alcohol. In these systems the filtration step is often the bottle-neck in the downstream process, with the separation of biopolymers or biomass from the liquid in a dead-end filtration being particularly difficult [2,3]. Dynamic filtration was used in order to reduce the fouling problem was used namely rotating disc filtration, which represents a further possibility for reducing the surface layer on the membrane in dead-end filtration [4-6]. In this study, a superimposed electric field induced a force on charged biopolymers in order to reduce the surface layer on the membrane. The use of an external electric field is a promising approach towards improving the permeate flux in crossflow filtration [7-9]. The new-typed electrofiltration indues high amount of permeates and makes the separation of biopolymers easier [10,11]. The aim of the present work was to investigate how to overcome the fouling in

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Table 1. Analyses on crude and 1,000 rpm-centrifuged fermentation broth

	Crude broth	Centrifuged broth
Ethanol (% (v/v))	6.5	5.0
PH 3.9	3.9	3.9
Acids (% (v/v))	1.0	0.9
Lactic acid (mg/L)	245	
Citric acid (mg/L)	200	
Aminoacid (% (v/v))	0.29	0.21
Protein (mg/L)	0.41	0.40

the process by means of electro-microfiltration experiments performed on a crude fermentation broth from the brewing industry, in comparison with microfiltered runs performed with the same broth.

MATERIALS AND METHODS

Materials

The fermented broth used in the present study was elaborated at the experimental unit. The composition of crude broth is reported in Table 1. Amylase was purchased from Pacific Chemical (Seoul, Korea). Yeast was obtained from Mido Chemicals (Daeku, Korea) and glucose was measured with glucose card test strip (Arkaray, Japan). Crushed rice was used as the fermentation material for the purpose of brewing. 300 g of crushed rice and 3 g of amylase were mixed with 1 liter of water, and the pH and glucose levels were measured every 12 h during the hydrolysis until the glucose level reached 5,000 mg/mL. Particle removal from the crude broth was obtained by 10 min of centrifuger at 1,000~4,000 rpm, using a Hanil model centrifuger (Hail Science MF 80 Model, Korea). This centrifuger is composed of 12 individual 15-mL tubes, and it was employed to purify the crude broth, which depends on the centrifugation speed.

Membrane and Microfiltration Experiments

The membranes used in the present study were materials provided by Millipore (USA). They had pore sizes of 0.45~5.0 μm and had 47 mm diameter-dimension. Membranes were prepared from a polyvinylidene fluoride membrane made by the so-called phase inversion technique and they were hydrophilic and hydrophobic in nature. Membranes rated average pore size was 0.4 μm and the maximum pore size was 0.5 μm.

Microfiltration experiments were performed with a laboratory-sized plant. Membrane purification apparatus was made by stainless steel and had pressure ranges between 0 and 10 kg_f/cm^2. Masterflex Easy-Load model (7518-00, USA) was used as the hydraulic pump and

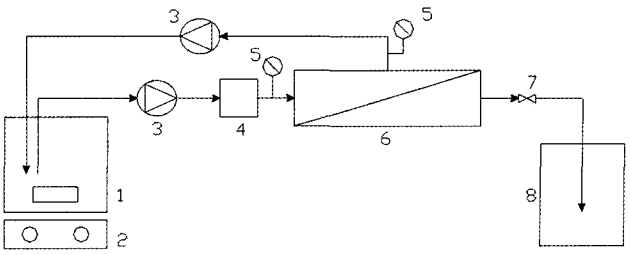

Fig. 1. Schematic diagram of the laboratory-sized apparatus. 1: electric power unit, 2: control box, 3: peristatic pump, 4: mixing tank, 5: metering pressure-gauge, 6: membrane apparatus, 7: on-off valve, 8: permeate collecting tank.

controlled the permeate flux. The experimental apparatus used in this work is shown in Fig. 1. The filtration area was 17.3 cm^2: three new membrane segments, hold in a polyvinyl carbonate device, were used for each experiment. The operating conditions were: filtered volume 700 mL, mean tangential flow velocity 2 cm/s, temperature 20°C and the flow circuit was constructed entirely of stainless steel. Pumping was provided by means of a variable speed gear pump, providing low shear to avoid denaturation of amino acid *etc.*. Transmembrane pressure was adjusted to 10 kg_f/cm^2. The permeation flux was determined by weighing and timing. At the beginning of each microfiltration experiment, the broth was filtered on the new membrane until the flux has reached a constant value.

Measurement

Turbidity measurements were performed with a Hach turbidometer (Loveland, USA) calibrated with turbidity standards of 0.1 NTU. True color was measured using the Hach (Loveland, USA) method with A 500 platinum-cobalt by centrifuging out the suspended materials in the speed of 1,000 rpm. The calibration is done in color at 455 nm units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as the chloroplatinate ion.

Alcohol was measured with gas chromatography (HP, USA) using a CBP-20 column. The operating condition was increased from 40 to 170°C by 7°C/min and then increased to 190 by 9°C/3 min. The temperature of the detector was maintained at 220°C and N_2 has was used as a carrier gas. Total acid was measured using a phenol phthalane indicator and titrated with 0.1 N NaOH. The concentration of the protein was measured by the Bradford method, using a kit supplied by Bio-Rad Lab. (USA).

Permeability in the Membrane

It is assumed that the pressure drop in the membrane ΔP_m is added to the pressure drop over the filter cake ΔP_c to give the overall pressure drop.

$$\Delta P = \Delta P_c + \Delta P_m \quad (1)$$

If the cake resistance R_c is considered in Darcy's law,

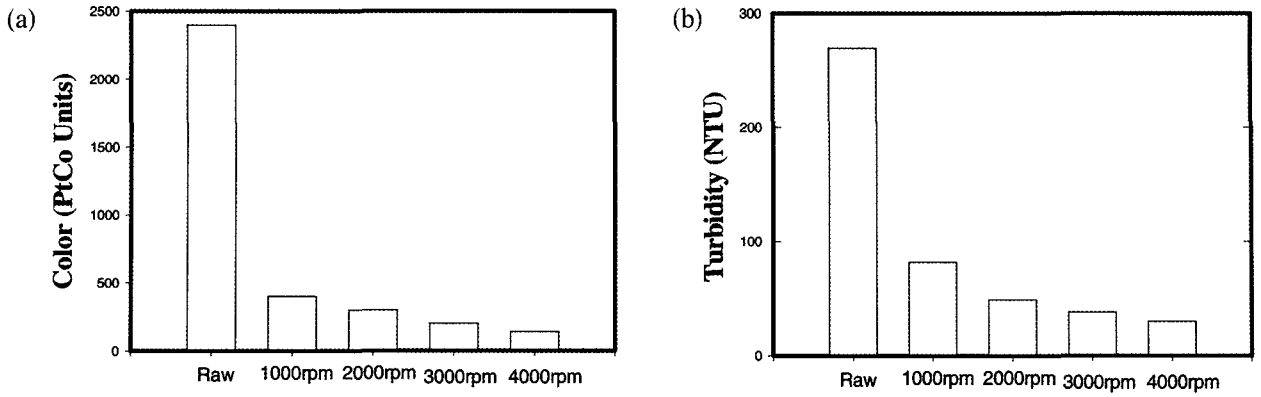

Fig. 2. Variation of centrifuged fermentation broth during centrifugation. (a) color variation depending upon the centrifugal speed, (b) turbidity variation depending upon the centrifugal speed.

where $R_c=L_c/k_c$:

$$\Delta P_c = \mu R_c \frac{dV}{dt}\frac{1}{A} \tag{2}$$

where L_c is the cake thickness and k_c is the cake permeability. Eq. (2) can be rewritten by substituting as follows [12].

$$\Delta P = \frac{\mu}{A}(R_c + R_m)\frac{dV}{dt} = \frac{\mu C\alpha}{A^2}V\frac{dV}{dt} + \frac{\mu}{A}R_m\frac{dV}{dt} \tag{3}$$

where α is $1/k_cC$, called the "specific resistance", and C is the cake volume fraction concentration. Eq. (3) is a straight line on a plot of filtration pressure against filtrate volume. Eq. (3) can be integrated using the following limits: zero filtrate volume at zero time, and V filtrate volume after time t. Thus,

$$\frac{t}{V} = \frac{\mu\alpha C}{2\Delta PA^2}V + \frac{\mu R_m}{\Delta PA} \tag{4}$$

Eq. (4) is a straight line, where t/V is dependent and V is the independent variable. Hence a graph of the experimental data points of t/V against V permits the calculation of the gradient and intercept of Eq. (4). The gradient and intercept are as follows:

$$\text{Gradient} = \frac{\mu\alpha C}{2\Delta PA^2}$$

$$\text{Intercept} = \frac{\mu R_m}{\Delta PA}$$

The permeability was measured in the pressure ranges of 1~10 kg_f/cm^2 using Eq. (3). The slope and intercept of Eq. (4) were then estimated using the experimentally obtained data in order to estimate the permeabilities and the resistances.

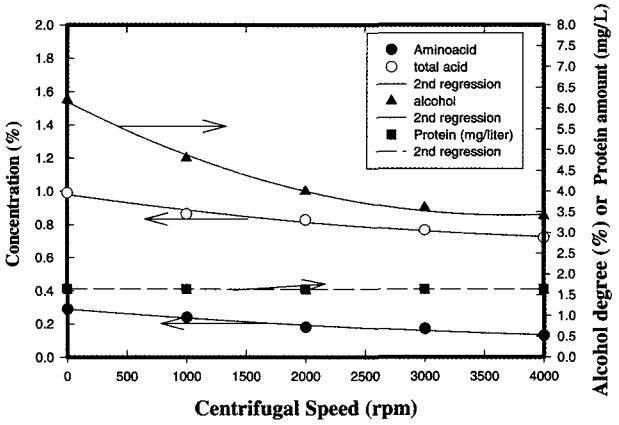

Fig. 3. Effect of centrifugation speed on the compositions of fermentation broth.

RESULTS AND DISCUSSION

Purification During the Centrifugation of a Crude Broth

Centrifugation only slightly improved performances of purification of the fermentation broth. Fig. 2 shows that the compositions inside the crude broth varied depending upon centrifugal speed. Color and turbidity were measured. Physical treatment by centrifugation contributed to the removal of suspended materials, and solid substances as a pretreatment of purification. Upon centrifugation, the turbidity of the broth changed significantly from 270 NTU of the crude broth to values inferior to 30 NTU of centrifuged broth, and concurrently the color changed from 2,400 PtCo color unit to values inferior to 140 PtCo color unit. The main reason for this can be attributed to the large removal of the microorganisms by centrifugation. As shown in Fig. 3, the alcohol and total organic acids were also decreased by the 1,000 rpm centrifugation, but further decreases were slight upon faster centrifugation.

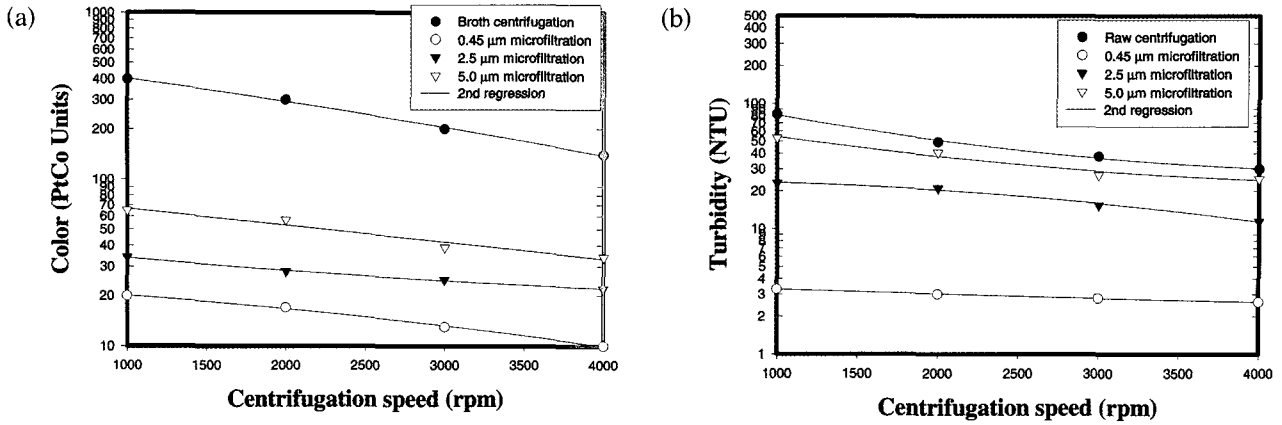

Fig. 4. Effect of pore size on membrane filtration using different centrifugation broths. (a) color variation depending upon the centrifugal speed, (b) turbidity variation depending upon the centrifugal speed.

From the experiment, the removal efficiencies of color were increased by 83.3, 87.5, 91.6, and 94.2% as the centrifuged speed increased to 1,000, 2,000, 3,000 and 4,000 rpm, respectively. With the same increases in centrifugation speed, the turbidity increased by 69.6, 81.8, 85.9, and 91.9%, respectively. The removal efficiencies of protein did not change much, but the removal efficiencies of the amino acid were 17.2, 37.9, 41.4, and 55.2% according to the above mentioned increases in centrifuged speed, respectively. Total acids had removal efficiencies of 12.7, 16.4, 22.7, and 27.3%, respectively and alcohol had removal efficiencies of 22.6, 35.5, 41.9, and 45.2%, respectively.

Purification During Microfiltration

Microfiltration experiments were repeated with the centrifugation broth. The solution of particles was removed from the centrifugation apparatus, and permeates were recovered from the microfiltration of the crude broth. The surface deposit was mainly made of organic aggregates, yeast and bacteria *etc.*, these made up a determinant part in cake formation. Centrifugation provides highly effective microfiltration performances. For example, the initial fouling rates were significantly decreased while the volumetric flow rate increased. However, microfiltration contributed to a decrease in the turbidity of the centrifuged broth from 82 NTU to values inferior to 3.3 NTU and to the color from 400 PtCo color units to values inferior to 20 PtCo color units as shown in Fig. 4 (a) and (b). The overall removal efficiencies during microfiltration with a pore size of 0.45 μm had 93.0~95.0% in the case of color and were 91.0~95.0% in case of turbidity depending upon the centrifugation speed. But these removal efficiencies were significantly different depending upon the pore sizes as shown in Fig. 4. The removal efficiencies during microfiltration with a pore size of 2.5 μm had 83.6~91.0% in the case of color and did 61.0~72.0% in case of turbidity. But the color and turbidity were significantly decreased by 75.7~83.0% and 16.0~35.4%, respectively during microfiltration with a pore size of 5.0 μm, respectively. In particular, the turbidities during microfiltration were greatly dependant on the pore sizes of the membrane.

When the trans-membrane pressure (TMP) increased, numerous microorganism and some aggregated organic matter were deposited on the front of the membrane front face, but this deposition remained insufficient to lead to a full coverage of the surface. The membrane experiment shows that the permeation was difficult to penetrate through the membrane having a 0.22 μm pore size even though the centrifuged speed increased by 4,000 rpm. However, the color and turbidity significantly decreased having a removal efficiency of more than 91% with the membrane having a 0.45 μm pore size. The membrane purification did not significantly vary irrespective of centrifuged speed. As a result, it was efficient to purify the broth with the membrane having a pore size of about 0.45 μm.

Purification During Electromicrofiltration

The applied electric field strength was 8~24 V. In this experiment hydraulic pressure was also applied over 2 kg_f/cm^2. It is apparent that there is a retentate layer on the anode-side of membrane, while on the cathode-side of membrane there is a permeate layer which is almost clear. If the pressure is increased, the highly compressible biomaterial's layers are compressed. This leads to the effect that an electric field acts along the cake layer. As the electric field increases, the biomaterial's migration velocity increases. So the electric field and therewith the electrophoretic velocity of the constituents close to the cathode-side of membrane is reduced at smaller total concentrations than in electrofiltrations with higher pressure. As the electric field increased, the permeate rate was also increased depending upon the size of the electric field as shown in Fig. 5. As a result, the permeate velocities were increased by 60.0, 95.5, and 133.3% as the electric field was increased to 8, 15, and 24 V, respectively. This indicates that the permeate velocity increased according to the direction of the electric field and the

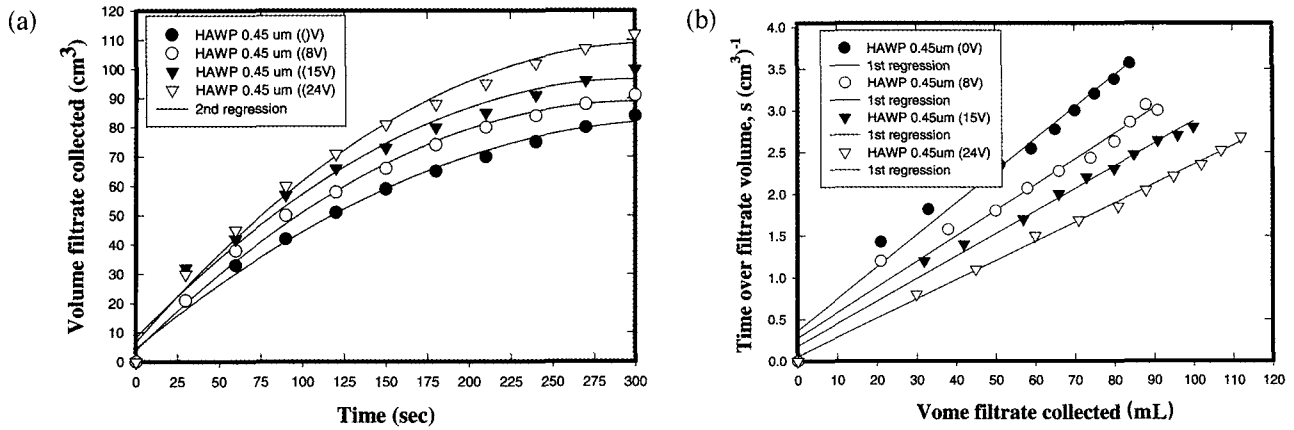

Fig. 5. Experimental results to investigate membrane performances in four different electric fields. (a) comparison of permeabilities in the membrane and cake layer, (b) comparison of resistances in the membrane and cake layer.

amount of permeate also increased.

The volume of the permeate as a function of time was measured by permeating the centrifugation-treated fermented broth through the micromicrofiltration membrane at a pressure of 3 kg_f/cm^2. The relationship between the permeation time t and V, the volume of the permeate divided by the membrane medium area, is shown in Fig. 5(a). The microfiltration membrane produced quite different results when compared with the electromicrofiltration membrane, as shown in Fig. 5(a). Even though the pore size of the micromembrane was 0.45 μm, the permeate flux substantially increased due to the electric field in the microfiltration membrane. The amount of permeation with the microelectrofiltration membrane was about two times greater than that with the microfiltration membrane. All the experimental results in Fig. 5(a) show a very good linearity, meaning that the rate of permeation remained constant. The slope of the straight line was the velocity of the permeation of the treated broth.

Based on the experimental results plotted in Fig. 5(b), R_m was calculated from the intercept of the extrapolated line for the filtration data with the y-axis [see Eq. (4)]. The resistance of the membrane filter calculated using this intercept is plotted in Fig. 5(b). The membrane resistance in the microelectrofiltration membrane at 8 V was significantly reduced by 23.9% to 0.1417 m^{-1} in comparison with R_m = 0.186 m^{-1} during microfiltration. Whereas the membrane resistances in the microelectrofiltration membranes at 15 and 24 V were reduced by 50.5 and 85.1% in comparisons with the microfiltration membrane. This indicates that the membrane resistances in the presence of the electric field were significantly reduced. The compositions of the permeate in the presence of an electric field can vary depending upon the level of the electric fields as shown in Fig. 6. The concentrations of proteins and amino acids in the fermentation broth were equally maintained during the microelectrofiltration, although they were significantly reduced by 45% by the microfiltration without the application of an electric field. Therefore, it is important for the microelectrofiltration membrane

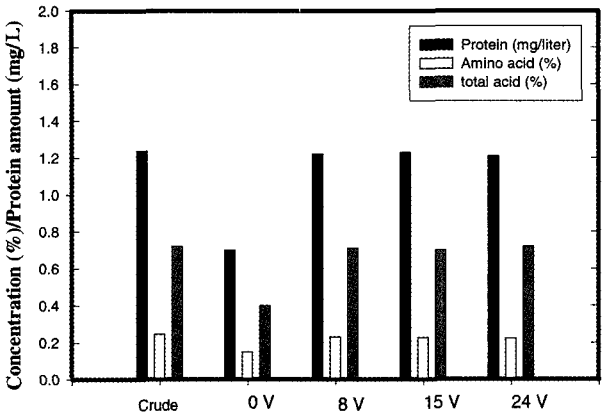

Fig. 6. Effect of electric fields in the variation of the compositions of fermentation broth.

to play a significant role in the transport of the solute in the process.

CONCLUSION

This work has shown that biopolymer dispersions take long filtration times in dead-end filtration to purify a fermentation broth. Measures that aim at reducing membrane resistance are not as effective as a reduction of the biopolymer cake resistance in a membrane. However, unexpected results were shown in our experiments with pressure electrofiltration. In this way the filtration time of a protein dispersion can be reduced from 100 min with pressure filtration down to a few minutes using electrofiltration. Furthermore, the electrofiltration with hydraulic pressure is an effective tool to influence the membrane processing. According to these findings electrofiltration is a promising alternative for the biopolymer recovery, and it may in many cases be superior to cross-flow filtration. The experimental results can be described as follows.

First, the membrane resistances in the microelectrofil-

tration membrane at 24 V were reduced by 85.1% in comparison with the microfiltration membrane.

Second, the proteins and amino acids of fermentation broth were equally maintained during the microelectrofiltration, although they were significantly reduced by 45% by the microfiltration without the application of an electric field.

Third, the permeate velocities were increased by 60.0, 95.5, and 133.3% as the electric field was increased to 8, 15, and 24 V, respectively.

Accordingly, since the electrofiltration membrane was provided more permeability, the present study experimentally demonstrates that the permeability inside a membrane can be controlled using an electric field.

Acknowledgement This study was supported by the research funds from Daejin University of 2004.

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[Received September 18, 2004; accepted December 1, 2004]