Fouling and Protein Adsorption

Effect of Low-Temperature Plasma Treatment of Membrane Surfaces

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ABSTRACT

Adsorption of proteins and the effect of the chemical nature of membrane surfaces on protein adsorption were investigated using ^{14}C-tagged albumin and several microporous membranes (polyvinilydene fluoride, PVDF; nylon; polypropylene, PP; and polycarbonate, PC). The membrane surfaces were modified by exposing them to low-temperature plasma of several different monomers (n-butane, oxygen, nitrogen alone or as mixtures) in a radiofrequency plasma reactor. Transients in the permeability of albumin solutions through the membranes and changes in flux of distilled water through the membranes before and after adsorption of albumin were used to investigate the role of protein adsorption on membrane fouling. The results show that the extent of adsorption of albumin on hydrophobic membranes was considerably more than that on hydrophilic membranes. The hydrophilic membranes were susceptible to electrostatic interactions and less prone to fouling. A pore-blocking model was successfully used to correlate the loss of water flux through pores of defined geometry.

Index Entries: Albumin; polymer membranes; permeability; radiofrequency plasma; radiochemical.

INTRODUCTION

Microporous membranes are commonly used in bioprocessing (1). The applications range from filtration of fermentation broth to filter-sterilization of liquid media. Hollow-fiber microporous membranes have potential for use as extraction devices also (2). Wang et al. (3) have demonstrated that such extractors may be used for a single-step purification and concen-

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tration of lactic acid from broth. However, the process was marked by significant reductions in the extractive flux of lactic acid over time. It was demonstrated that irreversible loss in permeation rate through the membrane was the primary reason for this reduction (4). Similar reductions in flux have been reported for many other applications also involving porous membranes (electrodialysis, reverse osmosis, ultrafiltration, and microfiltration) (5) and their negative economic impact on membrane industry has been estimated to be of the order of $500 million annually (6).

The flux declines in membrane operations are attributed to several distinct phenomena that include membrane compaction and degradation, concentration polarization, and membrane fouling (7). Concentration polarization has been widely studied and is relatively well understood (8). It can be minimized by an appropriate design of the membrane module and by appropriate selection of operating conditions (9). Membrane fouling, on the other hand, is poorly understood. Adsorption of solutes and their aggregates on the exterior and the interior membrane surfaces has been suggested to be the main reason for fouling (10–12). Hence, a number of authors have investigated surface properties of membranes and phenomenon of adsorption of solutes on the surfaces. One of the problems in relating the chemistry of membrane surface with solute adsorption using readily available membranes is that the membranes differ not only in chemistry but also in their pore structures. In these studies, it is desirable to use membranes with different chemistries but the same pore configurations. Low-temperature plasma treatment of surfaces permits creation of surfaces with different properties (13). Preparation of tailor-made membranes using low-temperature plasma treatment of surfaces has been suggested as a potential remedy for fouling (14–16). The objective of this work was to modify the surface characteristics of two polymeric membranes by low-temperature plasma treatment and then to investigate the relationship between surface chemistry, solute adsorption, and fouling characteristics of the surfaces.

Biological fluids contain several solutes such as proteins, carbohydrates, lipids, and their polymers, all of which have been suggested to be potential foulants (6). Of these, proteins have received considerable attention because of their common occurrence and complex interactions with themselves and with membrane surfaces (17–20). Hence, a protein (bovine serum albumin, BSA) was selected as a model foulant in this study.

MATERIALS AND METHODS

Globulin-free, lyophilized, and crystallized unlabeled bovine serum albumin was obtained from Sigma (St. Louis, MO). Its properties are well documented (21). ^{14}C-tagged BSA was obtained from American Radiolabeled Chemical (St. Louis, MO). The protein was dissolved in phosphate buffer (pH 7.0) prepared in high-purity water (distilled, ion-exchanged,

and deionized) and equilibrated for at least 3 h. The final solution was prefiltered through a 0.2-μm mixed cellulose-esters hollow-fiber capsule (Fisher, St. Louis, MO) and a 0.1-μm Calyx nylon filter capsule (MSI, Westboro, MA). Sodium azide (0.02%) was added to the albumin solution to prevent bacterial growth. The concentration of albumin in solution was measured by absorbance at 290 nm and by Commassie blue method. Radiolabeled BSA was tracked by counting the rate of disintegration in a liquid-scintillation counter (LS 7000, Beckman, Fullerton, CA) in presence of scintillation cocktail (ScintiSafe, Fisher).

Track-etched polycarbonate (PC) membranes of nominal pore diameters between 0.05 and 0.4 μm nominal pore sizes (diameter 47 mm) were obtained from Poretics (Livermore, CA). These membranes were reported to have straight nontortuous pores and low porosity; maximum deviation of pore axes from perpendicularity to the membrane surface is less than 30% and only up to 10% of the pores exist as doublets or triplets (22). Another hydrophobic membrane, polypropylene (PP), of nominal pore size 0.1 μm was obtained from Gelman Science (Ann Arbor, MI) and that of 0.45 μm nominal pore size from MSI (Westboro, MA). The 0.1 μm PP membrane was a stretched membrane with a very high membrane surface area (22 m²/g). The 0.45-μm PP membrane was a prefilter membrane made of intertwined fibers. Its surface area (2.3 m²/g) was low and similar to those of PC membranes (1.4–1.6 m²/g). The other membranes used were polyvinylidene fluoride (PVDF) from Millipore (Marlborough, MA), and naturally charged nylon membranes from Whatman (Fairfield, NJ). Both of these membranes were obtained in nominal pore sizes of 0.1, 0.2, and 0.45 μm. The PVDF membrane, as supplied, was hydrophilic because of grafting with polyhydroxypropylacrylate. Most of the available surface in the membranes was in pores (the ratio of total surface area to frontal surface area 595:1 for 0.1 μm PP membrane and 190:1 for 0.45 μm PP membrane); the corresponding values in PC membranes ranged from 10:1 to 12:1.

Fouling was quantified by measurements of permeation rates of clean water and of albumin solutions through modified and unmodified membranes in an Amicon 8050 stirred cell (Amicon, Beverly, MA). The experimental set-up is shown in Fig. 1. The transmembrane pressure in this study was varied between 0 and 12 psi. No aggregation of BSA has been reported during passage through microporous membranes at these transmembrane pressures (23). PC membranes (0.1 μm) were prewetted in order to reduce its wetting pressure in the operating range of pressures.

Protein adsorption on the surfaces was measured under static conditions using ^{14}C-bovine serum albumin. Equilibration between protein solution and the membrane(s) was conducted for 16 h followed by measurement of radioactivity on the membrane surface as well as in solution. The detection limit of the counting system and the effect of scintillation cocktail was established before the experiments. Surface area of the membranes was determined using a BET Sorptometer (Porous Material,
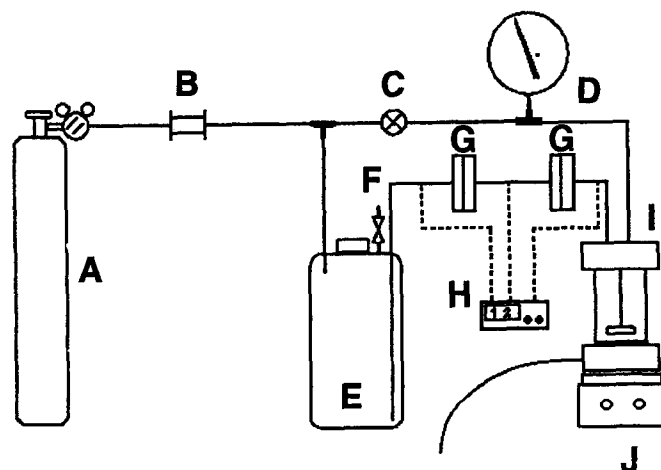
Fig. 1. Dead-end filtration set up: **(A)** nitrogen gas cylinder, **(B)** inline gas filter, **(C)** valve, **(D)** testgauge 0–15 psi, **(E)** reservoir, **(F)** pressure relief valve, **(G)** inline filters, **(H)** pressure tranducers, **(I)** stirred cell, **(J)** magnetic stirrer.
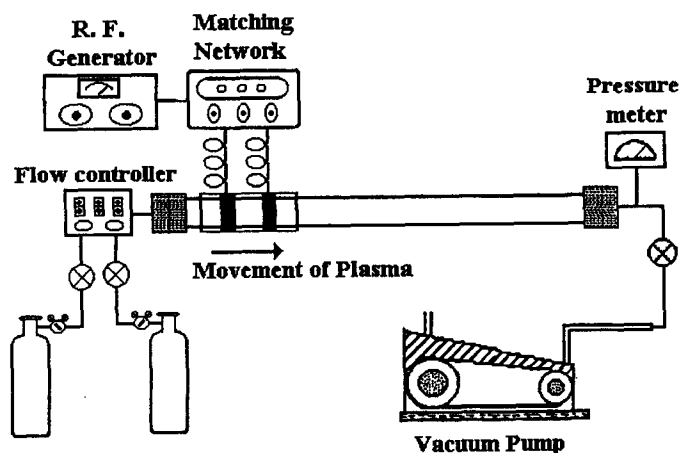
Fig. 2. Radiofrequency low-temperature plasma reactor.

Ithaca, NY) and argon gas. The data were analyzed using the Brunauer-Emmett-Teller analysis *(24).*

The low-temperature plasma treatment of membranes was conducted in a radiofrequency reactor, shown in Fig. 2. It consisted of a center tube (length 69 cm, od 3.9 cm, id 3.5 cm). The power was delivered in the form of 13.46 MHz radio frequency through two ring-shaped copper electrodes (width 1.3 cm, diameter 8 cm) located outside the reactor. The distance between the electrodes was set at 6 cm and the electrode assembly was placed symmetrically at the center of the tube. The unit was connected to a vacuum pump and pressure in the tube could be varied between 20 and 40 mtorr by manipulating a precision needle valve that also controlled the

flow rate of the monomers. The monomers used were oxygen and nitrogen without and with *n*-butane. Use of the different monomers (plasma) imparts specific groups and, therefore, different chemical nature to the membrane surface as discussed in the RESULTS AND DISCUSSION subheading without changing the pore structure of the surfaces. This permits elucidation of the effect of chemical nature of the surface on protein adsorption and fouling without undue effect of pore geometry. The membrane sample to be treated was placed at the center of the tube and in the middle of the electrodes. Proper placement of the membrane and the conditions for plasma treatment were established using silicon wafer as a substrate.

Surfaces were characterized by measuring the advancing contact angle of a distilled-water droplet on the membrane surface. The instrument used was a Gonimeter (Model G-I, Kernco, TX) connected to a video camera (Hitachi, Congers, NY). Scanning electron micrographs of the membranes were taken with an Amray scanning electron microscope model 1600 (Amray, Bedford, MA) at an acceleration voltage of 10 kV.

RESULTS AND DISCUSSION:

Protein Adsorption on Membranes

Equilibrium adsorption of BSA on the four membrane surfaces, measured under static conditions, is reported in Fig. 3. The adsorption on hydrophilic surfaces (PVDF and nylon) was measured in the membrane, as whole of the surface, including the pores, would be wetted under static conditions. For the hydrophobic surfaces, the pores would not be wetted under static conditions. Hence, adsorption was measured using flat sheets. In Fig. 3, the adsorbed concentrations have been normalized by the total (internal and external) surface areas that were calculated from BET adsorption isotherms of argon. The adsorption behavior on PVDF and nylon membranes was independent of pore sizes, suggesting that adsorption took place in the pores as well. This is to be expected as the size of the protein molecule ($11.6 \times 2.7 \times 2.7$ nm^3) was considerably smaller than the nominal pore size. For the hydrophilic membranes (PVDF and nylon), a simple calculation of monolayer adsorption of side-on BSA molecules shows that the adsorption was monolayer.

The adsorption behavior on PVDF, nylon, and PC surfaces was linear. Only on polypropylene surface did the adsorption show a saturation behavior. These results are in agreement with those of Persson and Nilsson *(25)* and of Gök et al. *(26)*. Yui et al. *(27)* also reported similar anomalies in adsorption of albumin on PP and polystyrene films; adsorption on PP showed a saturation behavior and that on polystyrene was linear in the whole concentration range. The adsorption on the hydrophilic membranes (PVDF and nylon) was considerably lower than that on hydrophobic membranes (PP and PC), as shown in Fig. 4. The higher amount of adsorption
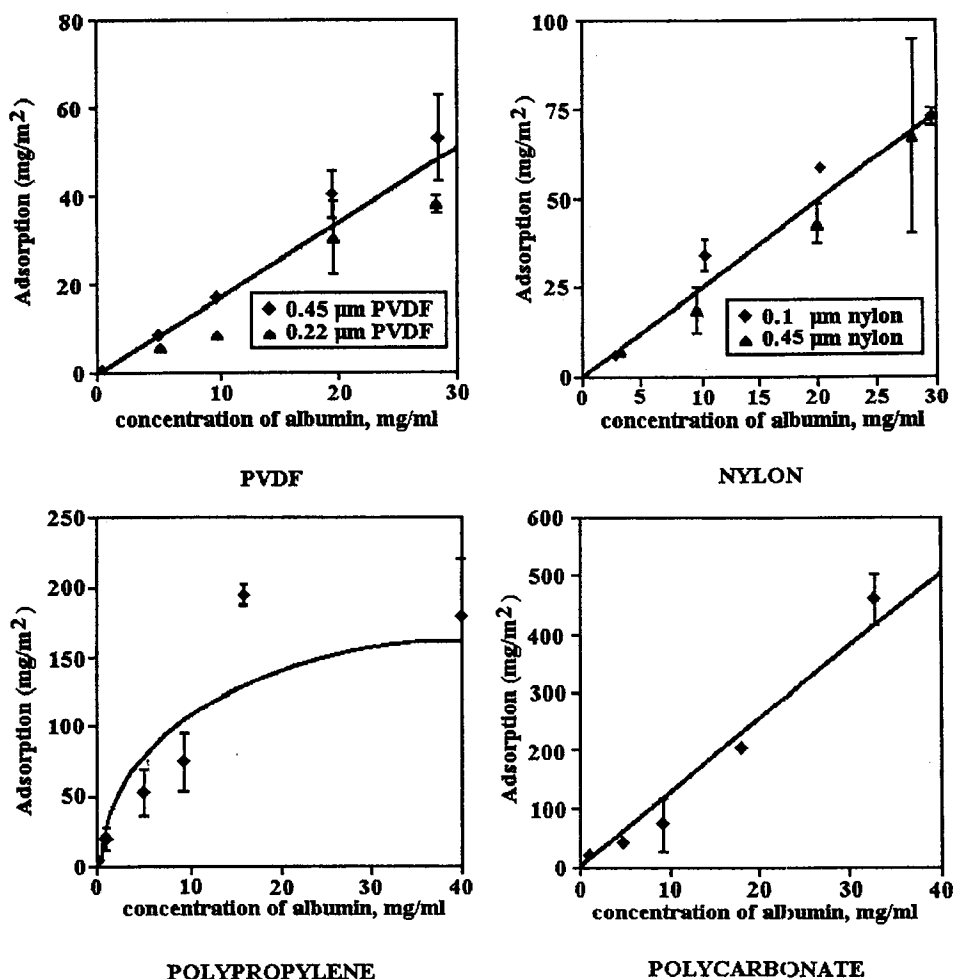
Fig. 3. Adsorption of BSA on untreated membranes.

on PP and PC is attributed to the hydrophobic nature of these surfaces. This too is in agreement with the observations of Persson et al. (28), Bowen and Gan (10) and Pitt (29). Higher adsorption of albumin on nylon than on PVDF suggests that charge interactions played a role in the case of hydrophilic surfaces. Nylon surface has a natural positive charge, whereas albumin (pI value of 4.8) was negatively charged at the operating pH (7.0). On the other hand, the hydrophilic PVDF membrane has been reported to have a zeta potential of −19.5 mV at pH of 7.0 (10). These results were confirmed by the effect of ionic strength of solution on the adsorption phenomenon (results not presented).

Fouling Behavior of Untreated Membranes

Membrane fouling was studied using two methods: by the transient changes in the flux of albumin solutions (50 mg/L and 1 g/L) through the
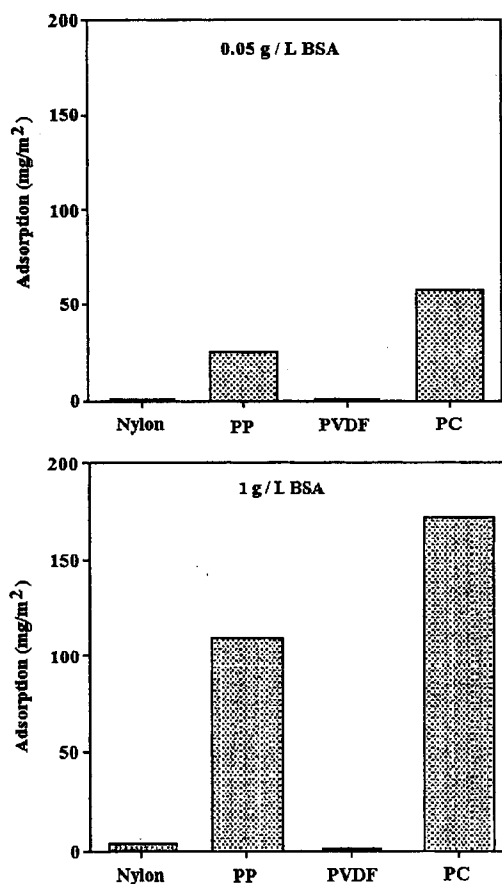
Fig. 4. Adsorption of BSA on untreated surfaces of 0.45-μm nylon and PVDF, and on flat PP and PC sheets (T = 24°C, pH = 7.0, 0.01 M phosphate buffer).

membranes in a dead-end stirred cell, and by the changes in water-flux through the membranes after static adsorption of BSA on the surfaces. It must be noted that the membranes were microporous and were not expected to show any rejection of protein at the pressure drops used in these experiments (1–10 psi). Water flux through the clean membranes were found to be invariant with time, suggesting that water arriving at the membrane surface had no particulates that could foul the surfaces.

1. The permeation flux of albumin solutions through the different unmodified membranes is shown in Fig. 5. The membrane with the least protein adsorption, PVDF (Fig. 4), showed no sign of flux-reduction for up to 10 h operation. As expected, the fluxes through larger pore-size membrane were higher than those through membrane with smaller nominal pore size. Several authors have reported that adsorption equilibrium is achieved relatively rapidly and is even faster under flow conditions. Hence, it may be concluded that protein solutions did not foul the PVDF membranes.
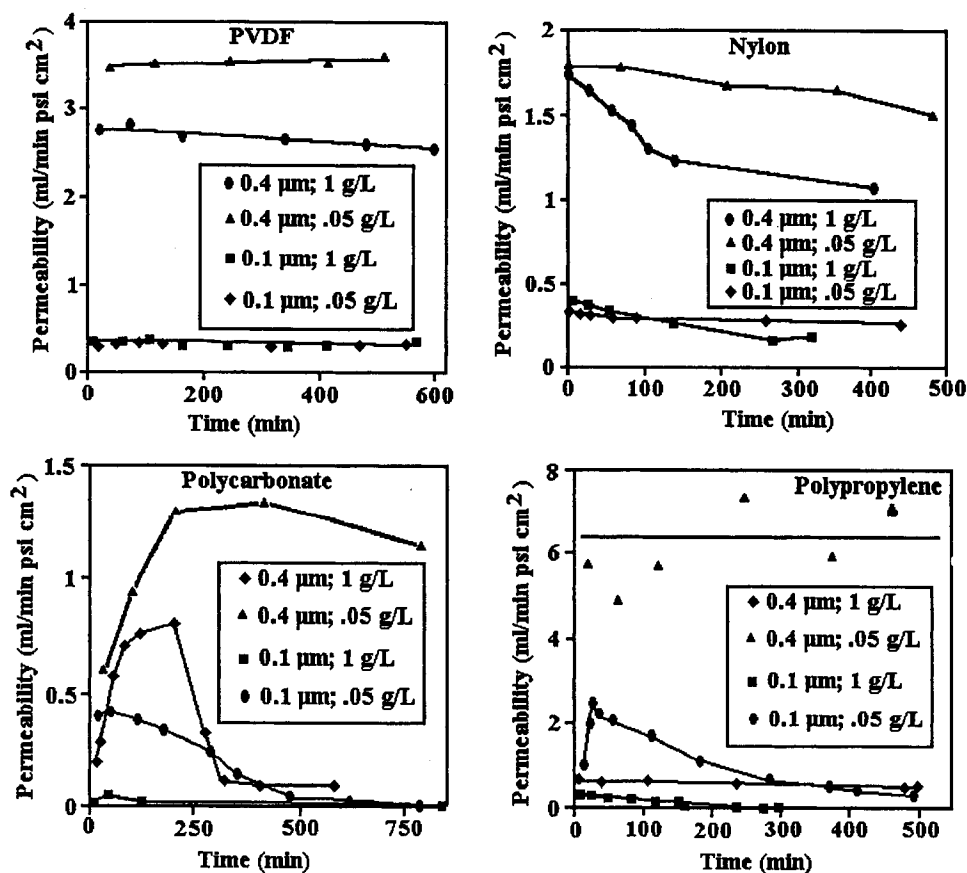
Fig. 5. Behavior of flux of albumin solution through untreated membranes.

It has been suggested by Persson et al. (28) that for these membranes, 10% of the pores control 90% of the solution flux. Hence, even if the smaller pores got fouled, the effect on flux may not have been observed.

Permeation flux through the nylon membranes (also hydrophilic) showed relatively more flux drop than for PVDF membrane for all the pore sizes. Although adsorption of BSA on nylon is also higher, it still was in the range of monolayer adsorption. Since Persson et al. (28) have suggested that monolayer sorption should result in only 3 to 4% flux reduction on a 0.2 μm nominal pore size membrane, the flux reductions observed here could signify induced aggregation of albumin (30).

For the hydrophobic membranes (PP and PC), the permeation flux increased with time before reducing again. For these hydrophobic membranes, initial adsorption of albumin perhaps increased the wettability of the surface, thus causing an increase in the flux. Further adsorption resulted in fouling and reduction in

flux. The increase was more dominant at lower concentration of BSA than at higher concentrations. The observations of the effect of albumin concentration on fouling were inconsistent with the published reports of Mueller and Davis *(23)* who found that fouling was more severe with low concentrations of protein than at higher concentrations. The 0.1-μm PP membranes did not show any initial increase in permeation flux since these were prewetted by 50% (V/V) isopropanol in water. This was necessary to get any flux at all through the smaller pore-size membrane under the operating pressures.

2. The losses in permeation flux of water through membranes, on which BSA was preadsorbed, were studied with PC membranes because of their well-defined pore geometry. Since PC is a hydrophobic membrane, adsorption of protein increases its wettability and thus the ease of passage of water through its pores. Hence, PC membranes coated with polyvinylpyrrolidone (PVP) were used in this part of study; membranes treated in such a manner were hydrophilic and permitted water flux through it at low transmembrane pressure drops, without any complications caused by changes in hydrophobicity of the membrane. The water flux through these membranes before and after BSA adsorption were measured and the ratio of the two water fluxes, defined as nominal flux, was plotted as a function of pore-size (Fig. 6). The changes in relative flux were analyzed by a pore-blocking model *(21)* that assumes that pore size is reduced by adsorption of monolayer of the protein molecules. In such a case, the steady-state expression of relative flux is given by

$$\left(\frac{J}{J_0}\right) = \left(1 - \left[\frac{\Delta r}{r_0}\right]\right)^4 \tag{1}$$

Here r_0 is the pore size before protein adsorption and Δr is the reduction in pore-size caused by to protein adsorption. Norde *(21)* has estimated the value of Δr for BSA 4.6 nm. Equation *(1)* is plotted in Fig. 6 as a solid line. An excellent agreement is seen between the predictions and the experimental observations. The dotted line in Fig. 6 represents a case in which protein adsorption would have no effect on water flux. It clearly establishes a relationship between adsorption and fouling.

Effect of Plasma Treatment on Membrane Fouling

The membranes were treated with oxygen, n-butane/oxygen, nitrogen, and n-butane/nitrogen plasma. The conditions for plasma treatments (power, duration, and flow rate) were optimized earlier. The effect of plasma-treatment on membrane fouling was studied only with hydropho-
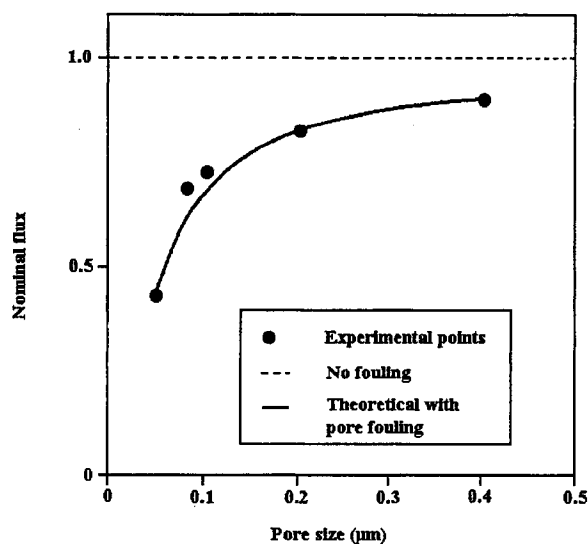
Fig. 6. Ratio of water flux through hydrophilic PC membranes after and before protein adsorption (nominal flux) as a function of pore sizes.

bic membranes (PP and PC) of large nominal pore sizes (0.4 or 0.45 μm). Fig. 7–9 show the SEM pictures of untreated and plasma-treated PP and PC membranes. Clearly, the membrane structures did not change appreciably. In all the cases, it was noticed that plasma penetrated the membrane pores and caused either the etching or the polymer deposition on the surface (including surface in the pores). Under the conditions of treatment, plasma treatment resulted in making the membrane surface more hydrophilic (i.e., advancing contact angle increased). The water fluxes through the plasma-treated and untreated membranes are listed in Table 1. Pore sizes of the PC membranes, estimated from SEM pictures are also listed in Table 1.

The corresponding water-permeation rates for PP and PC membranes are shown in Fig. 10. The untreated membranes showed a classic hydrophobic-membrane water-breakthrough response. Since the PP membrane has a wide pore-size distribution, water permeation rates through it show a highly nonlinear behavior as smaller and smaller pores are penetrated. It eventually becomes linear when all the pores get wet. PC membrane being of narrow pore-size distribution showed a linear increase in water flux after water breakthrough. The oxygen- and nitrogen-plasma-treated membranes showed water breakthrough even at zero transmembrane pressure drop, suggesting a hydrophilic nature of membranes thus treated. Butane-oxygen plasma treatment also resulted in a hydrophilic membrane and enhanced water flux, even though it was not as high as that for oxygen-treated membrane. The butane-nitrogen plasma resulted in even more hydrophobic membrane (advancing water contact angle increased) with considerably smaller pores. The reduction in pore-sizes was confirmed
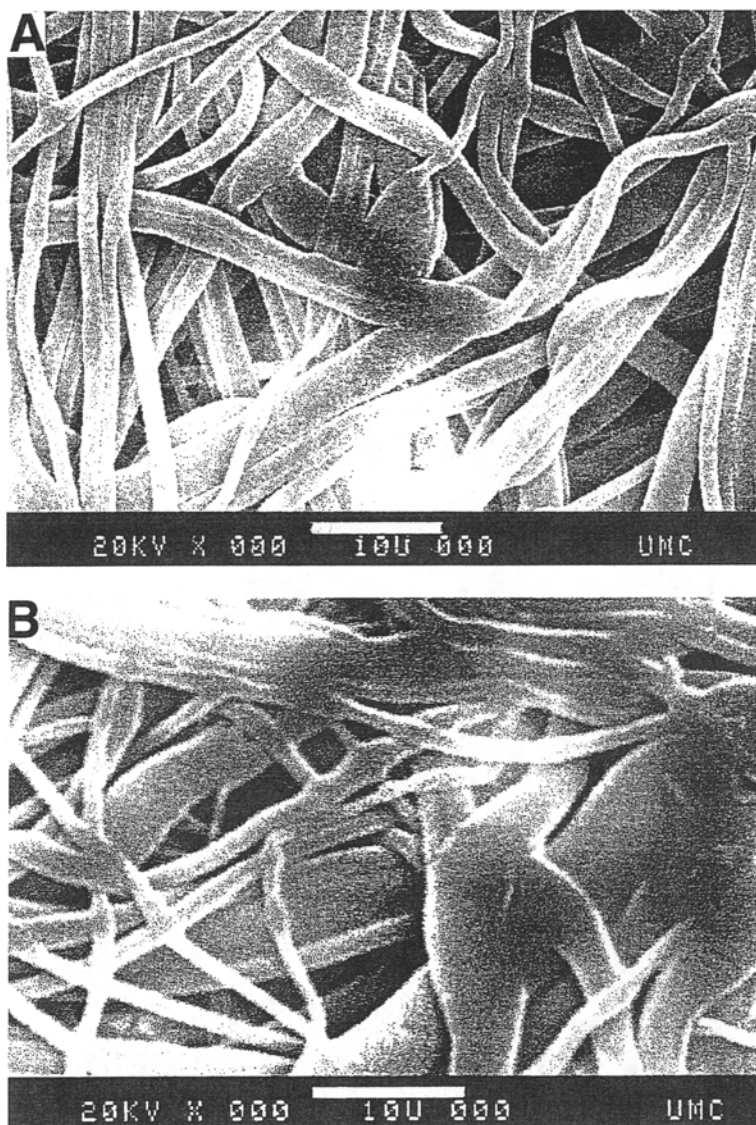
Fig. 7. SEM pictures of untreated and oxygen plasma treated 0.45-μm PP (4 W, F_{oxyg} = 0.7 sccm and 20 min).

by measurements of thickness of plasma-film deposited on silicon wafers under identical conditions. Butane-nitrogen plasma-treatment of silicon wafer produced a film thickness of 0.08 μm that corresponds well with a 0.15 μm reduction in pore size. Similarly, butane-oxygen treatment of silicon wafer produced a film thickness of 0.015 μm that would result in a pore-size reduction of 0.03 μm. This also agrees well with the observed reduction of 0.04 to 0.06 μm in SEM pictures.

The changes in permeability of albumin solution through treated and untreated membranes were also measured. These are shown in Fig. 11.
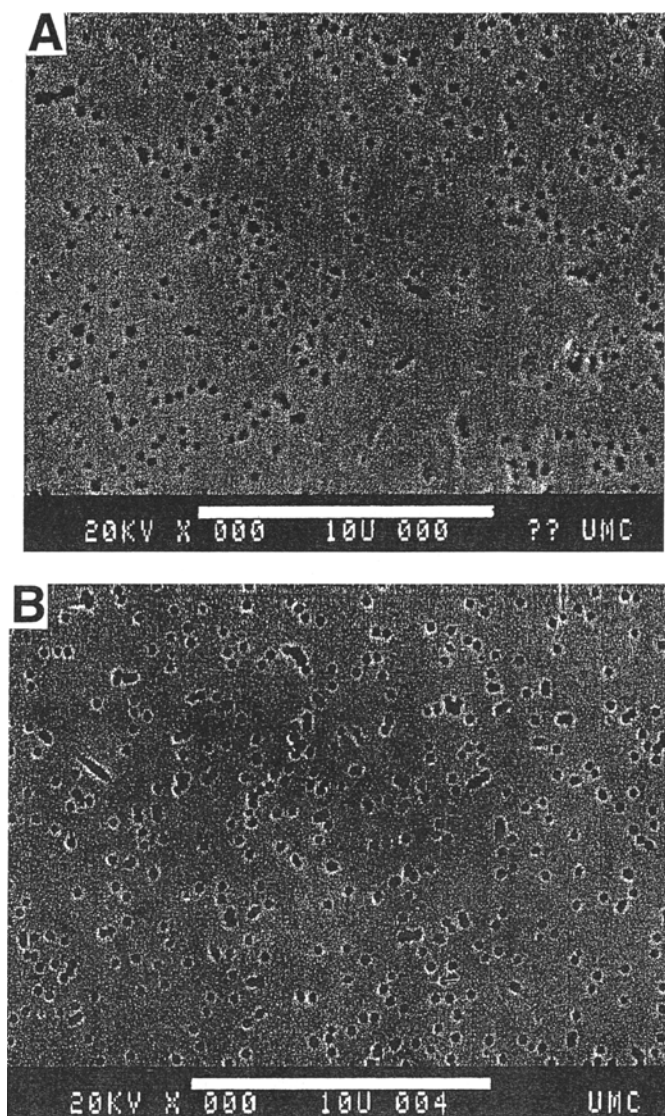
Fig. 8. SEM pictures on: **(A)** untreated 0.40-μm PC **(B)** oxygen plasma treated 0.40-μm PC (4 W, F_{oxyg} = 0.8 sccm and 20 min), **(C)** n-butane/oxygen plasma-treated 0.40-μm PC (20 W, F_{but} = 1.1 sccm, 40% oxyg and 20 min.).

Oxygen-plasma treatment of 0.45 μm PP membrane resulted not only in an increase in the solution flux, the flux did not undergo any reduction for 6 h of operation. On the other hand, the untreated membrane exhibited an initial increase in the flux, followed by a gradual decline that continued past the 6-h operation. The PC membranes also exhibited increases in flux after treatment with oxygen or butane-oxygen or nitrogen plasma. In all the cases, no transient increase was observed as was the case with untreated membrane. But the PC membranes demonstrated a continued reduction
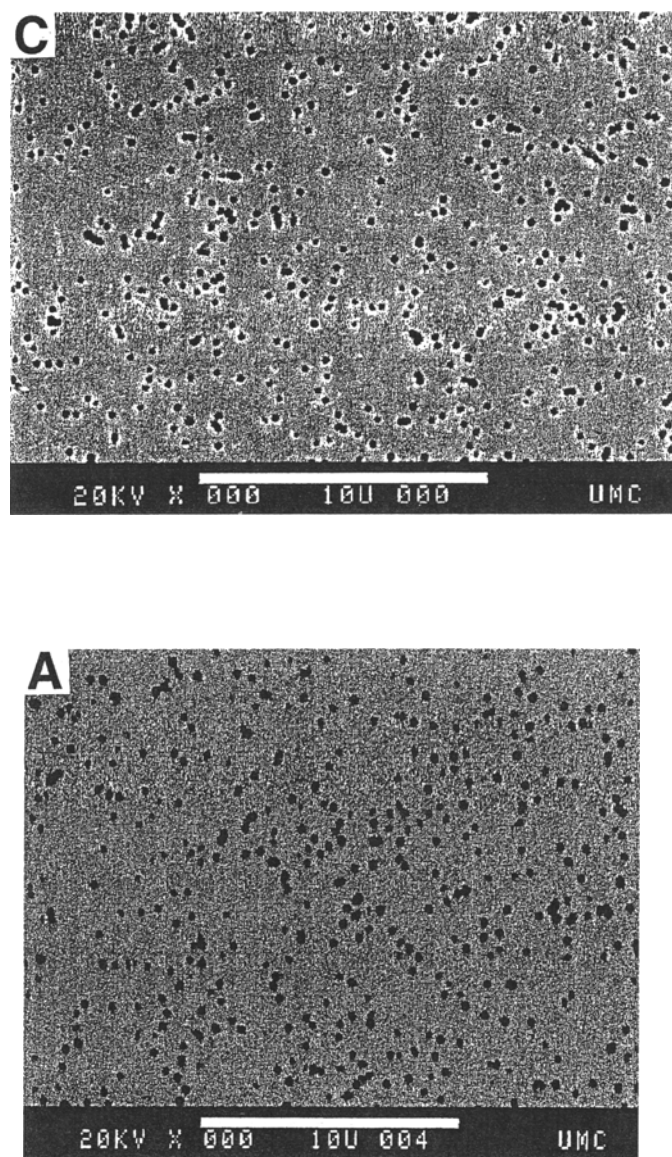
Fig. 9. SEM pictures of: **(A)** untreated 0.4-μm PC, **(B)** Nitrogen plasma treated 0.4-μm PC (7 W, 0.85 sccm nitrogen and 5 min), **(C)** *n*-butane/nitrogen plasma-treated 0.4-μm PC (20 W, 1.4 sccm *n*-butane, 48% nitrogen and 5 min).

of flux of albumin solution after 5 h of operation. Even after all the loss of flux, it was significantly higher than flux through the untreated membrane. The nitrogen-plasma treated membrane also showed a higher initial flux, but it dropped very rapidly. The butane-nitrogen-treated membrane had strongly shrinked pores as a result of treatment as well as higher hydrophobicity and flux through this membrane was never more than a fraction of fluxes in other cases.
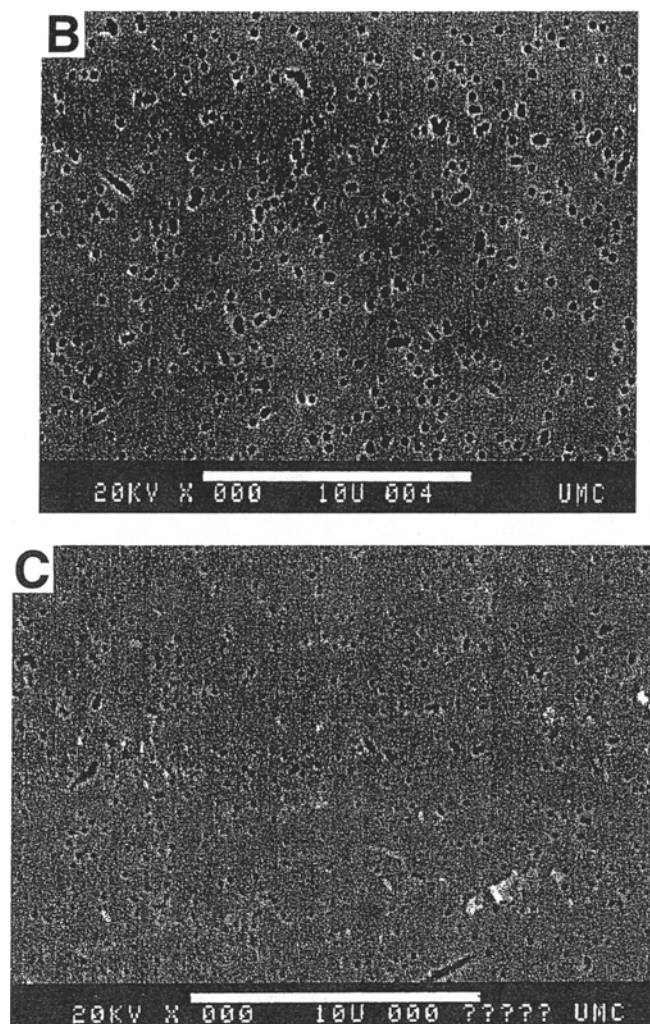
Fig. 9. *(continued)*

The stability of these treatments was tested by measurement of advancing contact angles of the treated membranes over an extended time period. The results showed that the treatments were stable.

CONCLUSIONS

Based on the results presented in this work, it may be concluded that hydrophilic membranes are least prone to fouling with bovine serum albumin present in fermentation broth. Under the conditions of operation, hydrophilic membranes were susceptible to electrostatic interactions. On the other hand, a strong absorption was observed on the hydrophobic membranes. A simple pore-blocking model could be used to correlate the flux loss through pores of defined geometry.

Table 1

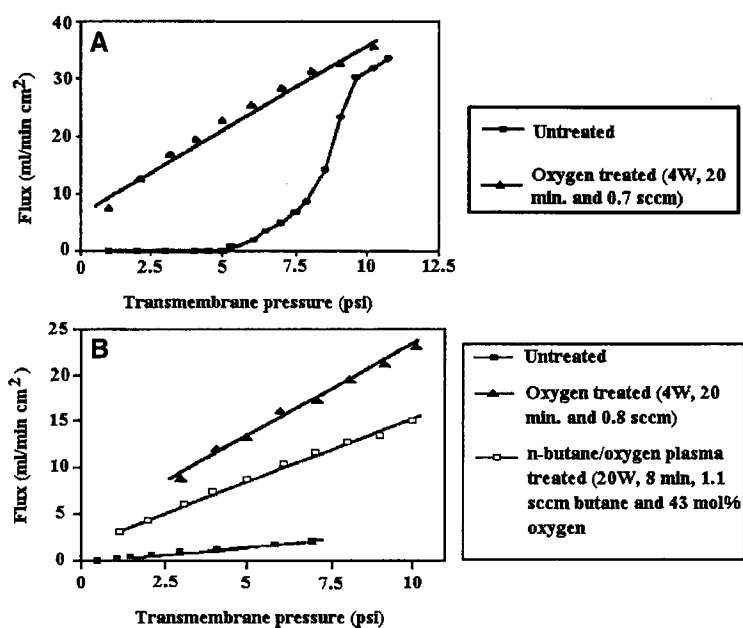| Membrane	Nominal pore size (n)	Treatment	Water flux mL/(min/ psi/cm^2)	Pore-size from SEM-pictures	Advancing water contact angle
PP	0.45	None	3.2		
		4W, F_{oxy} = 0.7 sccm, 20 min.	4.5		
PC	0.40	None	0.3	0.30–0.32	67°
		4W, F_{oxy} = 0.8 sccm, 20 min.	1.9	0.30	43.5°
			1.4	0.26	40°
		20W, F_{but} = 1.1 sccm, 40 vol % oxygen, 20 min.			
PC	0.40	7W, F_{nit} = 0.85 sccm, 5 min.	0.95	0.34	32°
			0.005	0.17	78°
		20W, F_{but} = 1.4 sccm, 48 vol % oxygen, 5 min.			

Fig. 10. Water permeation rates through untreated and treated PP (0.45-μm) and PC (0.40-μm) membranes as a function of transmembrane pressure drip.
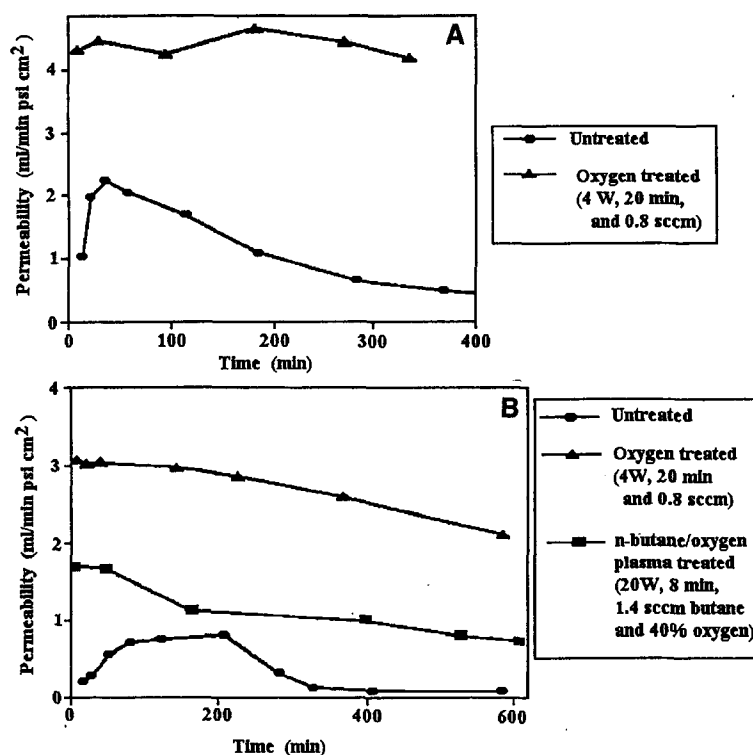
Fig. 11. Flux of albumin solution as a function of time for untreated and treated (A) 0.45-μm PP membrane and (B) 0.40-μm PC membrane.
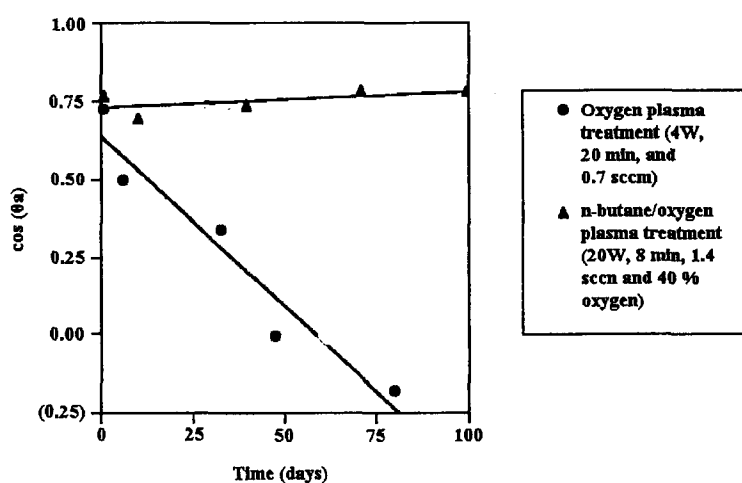

Fig. 12. Decay of advancing contact angle for oxygen plasma-treated 0.45 PP and *n*-butane/oxygen plasma treated 0.40 PC. For the first point in the decay of the oxygen plasma-treated membrane the advancing contact angle of a flat surface with the same treatment was used. Since the membrane was totally wet and no measurement of contact angle was possible.

Results of treatment of the hydrophobic membranes with oxygen, nitrogen, butane-oxygen, and butane-nitrogen plasma suggested that oxygen plasma and butane-oxygen plasma are the method of choice in reducing flux loss. Nitrogen treatment introduced amino groups on the surface. Under the pH of operation, these are positively charged and undergo charge interactions with the negatively charged protein and result in increased fouling. Butane-nitrogen plasma treatment failed to increase the flux and in making the surface more hydrophilic.

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