Bacterial Cellulose Membrane as Separation Medium

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SYNOPSIS

A thin membrane of bacterial cellulose (BC) obtained from *Acetobacter* culture was tested for its performance as a dialysis membrane in aqueous systems. The BC membrane showed superior mechanical strength to that of a dialysis-grade regenerated cellulose membrane, allowing the use of a thinner membrane than the latter. As a result, the BC membrane gave higher permeation rates for poly(ethylene glycols) as probe solutes. The cutoff molecular weight of the original BC membrane, significantly greater than that of regenerated cellulose, could be modified by concentrated alkali treatments of the membrane. The nature of the change at the ultrastructural level caused by the alkali treatments was studied by X-ray diffraction and scanning electron microscopy. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Acetobacter xylinum, a Gram-negative bacterium, produces cellulose extracellularly. This cellulose is formed as gel-like mass (pellicle) at the surface of the medium and can be purified by proper chemical treatments. This material has high crystallinity and large surface area and has been attracting attention as a new form of cellulosic material. The proposed application includes an acoustic vibrator taking advantage of its high elastic modulus[1,2] and an insoluble thicker/binder for foods and sheetlike materials.[3]

When a purified pellicle is dried on a flat substrate, a thin translucent cellulose membrane is formed. This membrane is expected to have unique properties because it consists of fine and continuous crystalline microfibrils, not like paper sheets or regenerated cellulose films. One possible application is molecular filtration such as dialysis or ultrafiltration. It has been proposed to use the bacterial cellulose as a dialysis membrane in nonaqueous systems.[4] On the other hand, regenerated cellulose membranes have been widely used as a dialysis membrane in aqueous systems, where chemical stability and low toxicity of cellulose are preferable properties, especially in applications for labile biological systems.

This study aimed at elucidating the basic characteristics of bacterial cellulose membrane as molecular separation medium in aqueous conditions, together with modifying the structure of the membrane by chemical treatments for controlling its molecular permeation characteristics. The tests were conducted in the dialyzing mode, i.e., without pressurizing the primary-side solution, by using a series of poly(ethylene glycols) [poly(ethylene oxide)] as probe solutes.

EXPERIMENTAL

Bacterial Cellulose (BC) Membrane

Thirty milliliters of sterilized Schramm–Hestrin medium[4] was placed in a plastic Petri dish (inner diameter, 87 mm) and was inoculated with *Acetobacter xylinum* (ATCC 23769). Immediately after inoculation, the medium was gently but thoroughly mixed by swirling the dish so that the cells were distributed uniformly. The culture was statistically incubated at room temperature for 5–7 days, until the liquid medium was filled with cellulose pellicle.

The harvested pellicle was rinsed with distilled water and soaked in 1% NaOH for 24 h for removing the incorporated cells and culture medium components, then thoroughly washed with distilled water

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to neutrality. The purified pellicle was placed on a glass plate and allowed to dry under ambient conditions. The formed membrane was removed from the plate by soaking in distilled water and then subjected to the permeation tests.

The thickness of the dry membrane was determined by a caliper to be 2.0 μm, which was close to the value calculated from its weight by assuming a density of 1.5 g/cm^3.

Concentrated Alkali Treatment

The cellulose membrane was treated with 20% NaOH, either by immersing the dry membrane in the alkali solution or by applying the alkali solution to the central part of horizontally placed dry membrane attached to the glass plate, leaving the peripheral area dry. In the latter way, the membrane was kept attached to the substrate and in-plane shrinkage of the membrane was prevented. In either method, the sample was treated with 20% NaOH for 20 min, thoroughly washed with distilled water, and dried at room temperature while being attached to a glass plate. The membrane was then detached from the substrate by wetting with distilled water and set to the permeation testing cell.

Regenerated Cellulose Membrane

A commercial regenerated cellulose (cellophane) membrane of a dialysis grade (Biomed Instrument, Chicago; nominal pore size, 1.5 nm) was tested for permeability for comparison. The thickness of the dry membrane was determined by a caliper to be 12.0 μm. This sample was also treated with 20% NaOH as above, but without fixation.

Swelling with Copper–Ethylene Diamine Solution

Eight times diluted copper–ethylene diamine solution (the standard solution being prepared according to Tappi Standard T254hm-85) was applied to BC membranes for 60 s as the swelling agent, similarly as in the alkali treatment described above.

Hydrothermal Treatment

The dry BC membrane attached to a glass plate was treated in an autoclave at 121°C for 10 min.

Permeability Measurement

The membrane was clamped between a pair of flanged glass cylinders (Fig. 1). Each side of the membrane was filled with 25 mL of distilled water,

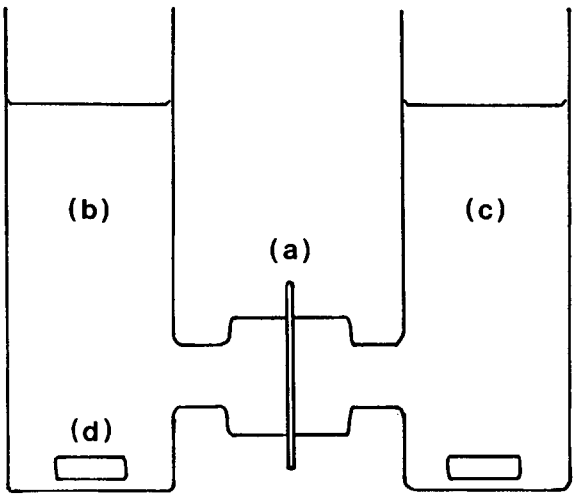


Figure 1 Schematic drawing of apparatus for permeation measurement: (a) sample membrane; (b) probe polymer solution (primary side); (c) pure solvent (water) to receive permeated solutes (secondary side); (d) magnetic stirring bar.

which could be continuously stirred by a magnetic bar. The area of membrane contacting the liquid was 3.14 cm^2.

A certain amount of a mixed poly(ethylene glycol) solution was added to one side (primary side) of the membrane to start the measurement. The subsequent change in the probe concentration was determined by taking 0.5–1 mL of the liquid from the other side (secondary side) of the membrane and analyzing it with an aqueous SEC system (TSK 4000PW + 2000PW, 7.5 mm i.d. × 600 mm each).

The effect of the change in liquid volume was compensated by removing the same amount of liquid from the primary side at the same time. The accuracy of this method was confirmed by directly measuring the concentration of single solutes in the secondary side by continuously recirculating the solution through a differential refractometer cell (Waters R-403).

Probe Solutes

The following fractions of poly(ethylene glycol) were used as probe solutes: triethylene glycol, MW = 150; poly(ethylene glycol), $\bar{M}_w$ = 300, 600, 1540, 4000, 6000, and 20,000. These were dissolved in distilled water to make a mixed solution of 1% (wt/wt) each.

X-ray Diffraction Analysis

The change in crystal structure of the BC membrane by alkali treatments was studied by X-ray diffrac-

tion. The diffraction pattern was recorded by a JEOL JDX-5B diffractometer by the reflection method with a CuKα line.

Scanning Electron Microscopy

The morphology of the BC membrane was examined by a Hitachi S4000 scanning electron microscope at 10 kV, by coating the specium with platium. The specium for this purpose was prepared by freeze-drying the water-soaked BC membrane samples to preserve the swollen state as much as possible.

RESULTS AND DISCUSSION

Figure 2 shows the molecular weight (MW) dependence of permeation rate of original BC and the alkali-treated samples thereof. The permeability of untreated BC membrane is the highest and its cutoff MW is above 20,000. The alkali treatments of this membrane without fixation resulted in significant decreases in both overall permeability and cutoff MW. Since the alkali treatment causes strong swelling and in-plane shrinkage of the membrane, the observed change seems to reflect the effects of its compaction and thickening in the course of swelling and drying.

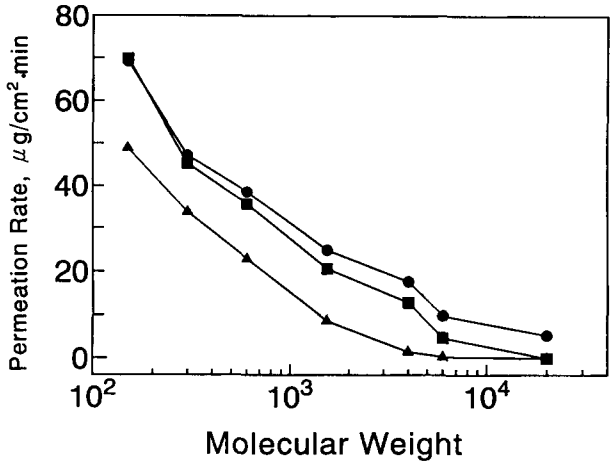


Figure 2 Molecular weight dependence of permeation rate of original BC membrane and samples treated with 20% NaOH with or without fixation: (●) original BC membrane; (■) alkali-treated BC membrane with fixation; (▲) alkali-treated BC membrane without fixation. Permeation rate is expressed as the amount of solute [poly(ethylene glycol)] that permeates the unit area of the membrane per unit time at a concentration difference of 1% (wt/wt) (same for Figs. 3, 6, and 7).

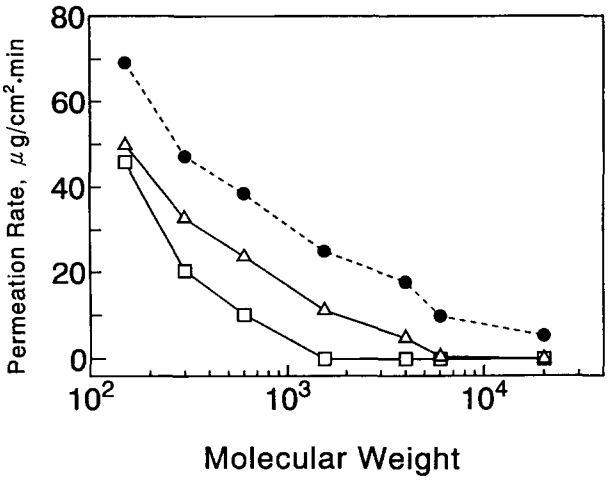


Figure 3 Molecular weight dependence of permeation rate of dialysis-grade cellophane membrane and alkali-treated sample: (□) cellophane; (△) alkali-treated cellophane; (●) original BC membrane.

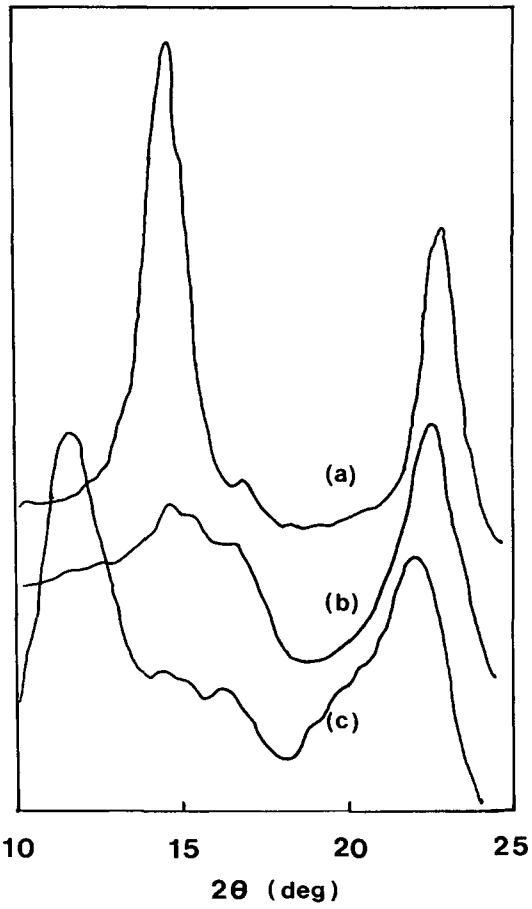
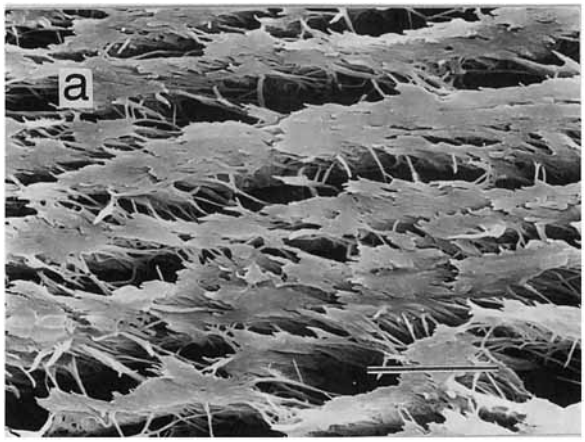


Figure 4 X-ray diffraction diagrams of BC membranes: (a) original BC membrane; (b) treated with 20% NaOH with fixation; (c) treated with 20% NaOH without fixation.

The permeability curve of the BC membrane treated with alkali with fixation is close to that of the original membrane, but the cutoff MW is now well defined at below 20,000 and these features can be understood as to reflect the effect of the fixation, which prevents swelling and thickening of the membrane. Still, the possibility of controlling permeability at high molecular weight ranges deserves attention.

On the other hand, the commercial cellophane membrane gave significantly lower permeation rates than did the original BC membrane, with the cutoff MW of ca. 1600 (Fig. 3). The difference is considered



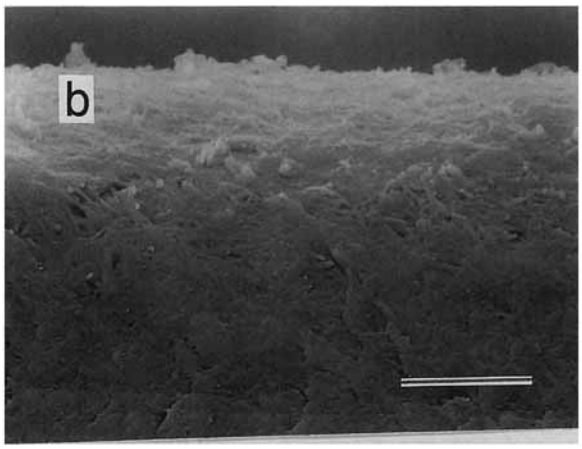


Figure 5 Scanning electron micrographs of razor-cut cross sections of (a) original BC membrane and (b) alkali-treated (fixed) membrane, both prepared by freeze-drying from the water-soaked state. The direction of surface is horizontal in both (a) and (b). Scale bars, 3.0 μm.

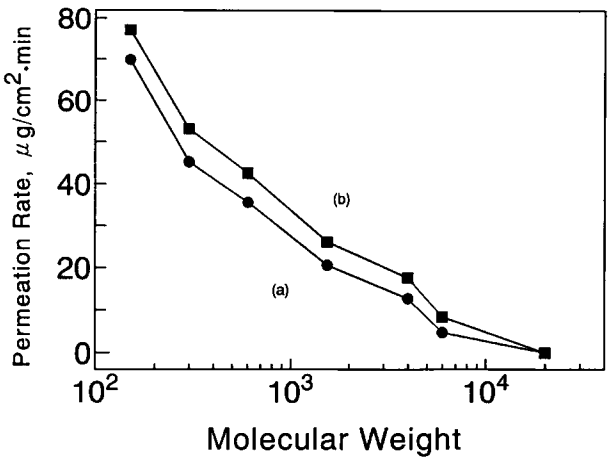


Figure 6 Molecular weight dependence of permeation rate of alkali-treated (fixed) BC membrane: (a) before and (b) after hydrothermal treatment.

to arise from the difference in thickness (2.0 vs. 12 μm) and the membrane structure. Also notable is the difference in the effect of alkali treatment, in that the alkali treatment of cellophane resulted in a considerable increase in permeability. The difference was also noted in the appearance of membranes during the alkali treatment: While the BC membrane showed significant shrinkage when it is treated without fixation, the cellophane membrane showed slight expansion when immersed in the alkali solution without fixation.

These features apparently reflect the difference between the crystal structures of the two cellulose membranes. Severe shrinkage is usually observed in mercerization of the native cellulose and the phenomenon is considered to result from some fundamental rearrangements of cellulose molecules.[5] On the other hand, the cellophane is composed of cellulose **II** crystallites and amorphous regions and, therefore, is considered to undergo no basic changes in the crystal structure by the alkali treatment. The change in permeability of cellophane by the alkali treatment seems to result from some loosening effect on the matrix structure.

Figure 4 shows the X-ray diffractograms of the original BC membrane and the alkali-treated samples. The original BC shows a typical pattern of cellulose **I** [Fig. 4(a)], with a strong planar orientation of the $(1\bar{1}0)$ lattice plane (the indexing according to Ref. 6) parallel to the surface of the membrane. The sample treated with alkali without fixation was found to be completely converted (mercerized) to cellulose **II** [Fig. 4(c)]. In contrast, the sample treated with alkali with fixation showed a relatively small change in the diffraction pattern, the most

notable change being the loss of planar orientation of (1$\bar{1}$0) plane [Fig. 4(b)].

For investigating the nature of ultrastructural change by the alkali treatment with fixation, water-soaked and subsequently freeze-dried BC membrane samples were examined by scanning electron microscopy (Fig. 5). The morphologies of these samples were remarkably different from each other: whereas the original BC membrane is composed of many lamella-like layers having wide gaps in between, the alkali-treated membrane has a densely packed structure. This difference could be noted during handling the samples for permeability measurements: The original membrane was more highly swollen and softer than the latter. Thus, the alkali treatment seems to have a compacting effect to the BC membrane, without changing the basic structure of cellulose **I.**

The hydrothermal (autoclave) treatment of the BC membrane was attempted with the intention of both enhancing its mechanical strengths and modifying the porous structure. No difference of the permeability was observed between the original BC membrane and the treated one (no figure shown here). In contrast, the hydrothermal treatment brought about a slight increase in the permeability, while keeping the same cutoff MW, for the alkali-treated BC membrane (Fig. 6). Since the hydrothermal treatment seems to cause rearrangement of unordered cellulose molecules, [7] the different permeability behavior between the original BC membrane and the alkali-treated one after the hydrothermal treatment is considered to reflect the difference in crystallinity of the two membranes.

The effect of the swelling of the BC membrane by cellulose solvent was examined using a diluted copper–ethylene diamine solution. The concentration of the solution had to be limited to below $\frac{1}{8}$th of the standard-strength solution, because the higher concentration resulted in complete dissolution. The treatment with this diluted solution actually caused a decrease in permeability of the BC membrane, but the effect was not remarkable (Fig. 7). The treatment, on the other hand, caused significant loss of mechanical strength and was not found useful for the present purposes.

In conclusion, the present results indicated that the BC membrane could be used as a molecular separation medium in aqueous environments, with

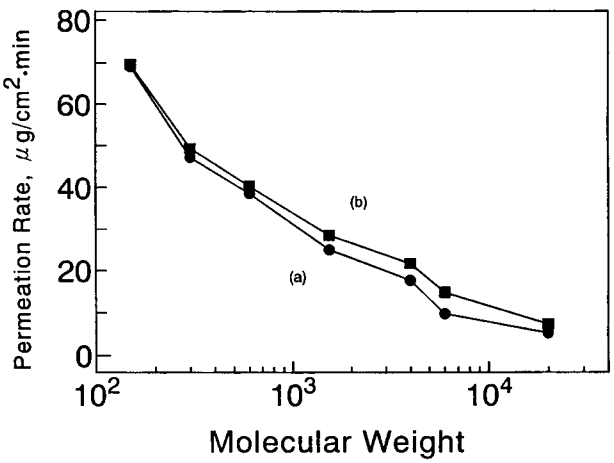


Figure 7 Molecular weight dependence of permeation rate of (a) original and (b) copper–ethylenediamine-treated BC membranes.

greater cutoff molecular weights than those of the regenerated cellulose membrane. Though its permeability for unit thickness is lower than that of regenerated membrane, higher permeation rates could be achieved by availability of a thinner membrane with sufficient mechanical strength. The permeation characteristics of the BC membrane could be effectively modified by concentrated alkali treatment, the feature being basically different from that of regenerated cellulose membrane.

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