Novel Nanoporous Membranes from Regenerated Bacterial Cellulose

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ABSTRACT: Bacterial cellulose (BC) in an NaOH/urea aqueous solution was used as a substrate material for the fabrication of a novel regenerated cellulose membrane. The dissolution of BC involved swelling BC in a 4 wt % NaOH/3 wt % urea solution followed by a freeze–thaw process. The BC solution was cast onto a Teflon plate, coagulated in a 5 wt % $CaCl_2$ aqueous solution, and then treated with a 1 wt % HCl solution. Supercritical carbon dioxide drying was then applied to the formation of a nanoporous structure. The physical properties and morphology of the regenerated bacterial cellulose (RBC) films were characterized. The tensile strength, elongation at break, and water absorption of the RBC membranes were 4.32 MPa, 35.20%, and 49.67%, respectively. The average pore size of the RBC membrane was 1.26 nm with a 17.57 m^2/g surface area. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 292–299, 2008

Key words: biomaterials; membranes; nanostructure

INTRODUCTION

Cellulose is one of the most important structural elements in plants as the main component of cell walls. Cellulose from plants is considered impure, containing many kinds of complex carbohydrates. Nata de Coco is bacterial cellulose (BC) synthesized by *Acetobacter xylinus* with coconut water as a liquid medium. BC presents significant advantages over plant-derived cellulose with its structural and mechanical properties. Unlike cellulose from plants, BC is chemically pure and free of lignin and hemicellulose. BC has high crystallinity and a high degree of polymerization.[1] Plant-derived cellulose and BC have the same chemical composition but different structures and physical properties. The BC network structure comprises cellulose nanofibrils 3–8 nm in diameter, and the crystalline regions are normal cellulose I.[2,3] Together, the mesh of these fibrils forms a gelatinous membrane.[4,5] BC has mechanical properties that are superior to those of plant cellulose and many synthetic fibers. Young's modulus of BC is approximately 4 times greater than that of general organic fibers.[6] In comparison with the cellulose from plants, BC has a higher water holding capacity, higher crystallinity, and higher tensile strength.[7] Because of these unique properties, BC can be used in a variety of different applications such as food matrices, diet food, and high-performance acoustic diaphragms for audio speakers.[6] For medical uses, BC has been applied as an artificial skin for patients with burns and ulcers,[8,9] as a temporary substitute for animal skin,[9] and as artificial blood vessels for microsurgery.[1] BC is also a promising material for use as a matrix of palladium membranes in fuel cells[10] or as dialysis membranes.[11] BC films demonstrate superior mechanical strength to that of regenerated cellulose membranes, and this allows the use of BC in the form of a thinner dialysis membrane with a higher permeation rate.[11]

In recent years, regenerated cellulose membranes from plants have been widely used in membrane separations such as dialysis, ultrafiltration, and reverse osmosis. The strong interchain and intrachain hydrogen bonding involved in the crystalline regions is known to make reactions and dissolution of cellulose difficult. The current industrial routes for dissolving cellulose usually require strong alkali conditions that lead to pollution problems with hazardous wastes. Consequently, environmental concerns are driving the efforts to find new solvents to dissolve cellulose. Recently, solutions such as NaOH/urea and NaOH/thiourea aqueous systems have been successfully applied to the dissolution of plant cellulose.[12–18] Although studies of regenerated cellulose from plant-derived cellulose have been extensively investigated and developed by many

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researchers,[12–21] only very few studies, if any, have been conducted with BC as a source of cellulose.

Because of the unique properties of BC, in this study we attempted to develop a new regenerated cellulose membrane from BC in an NaOH/urea aqueous solution. The structure, pore morphology, tensile strength, components, and water vapor permeability of the developed membrane were then investigated and compared with those of the biosynthesized BC film. On the basis of the nanostructure of the BC film together with its good properties, particularly its chemical stability and biocompatibility, possible applications of this novel regenerated bacterial cellulose (RBC) membrane to nanoseparation in chemical processes and in medicine are expected.

EXPERIMENTAL

BC

BC was kindly provided by the laboratory of Pramote Thamarat (Institute of Research and Development of Food Product, Kasetsart University) as sheets of Nata de Coco. Nata de Coco is a gel-like cellulose pellicle formed by *A. xylinus* cultures on the surface of a medium containing 0.5% ammonium sulfate, 5.0% sucrose, and 1.0% acetic acid in coconut water. The sheets of BC were purified by washing with deionized (DI) water, then were treated with 1 wt % NaOH at 35°C for 24 h to remove bacterial cells, and were rinsed with DI water until the pH was 7. Afterward, the BC film was air-dried at room temperature (30°C) and stored in a plastic film before use.

Preparation of the membranes

Dissolution of BC in an NaOH solution

To determine the solubility of BC in an NaOH aqueous solution, 3 wt % BC was cut into small pieces (0.3×0.3 cm^2), dispersed in 1–10 wt % NaOH aqueous solutions, and stirred for 10 min at room temperature to obtain a BC slurry. The slurry was then cooled to −5°C in a freezer and held at −5°C until it became a solid frozen mass for 12 h. The frozen solid was then allowed to thaw and was stirred extensively at room temperature to obtain clear BC solutions. The insoluble parts were isolated by centrifugation at 6000 rpm and 10°C for 30 min and then neutralized with 1 wt % NaOH, 0.5 wt % NaOH, 0.5 wt % acetic acid, and distilled water, respectively. After that, the insoluble parts were dried in a vacuum oven at 35°C for 24 h and weighed. The experimental studies were carried out in triplicate. The solubility (S_0) was calculated with the following formula:

$$S_0 = \frac{w_0 - w_i}{w_0} \times 100\%$$

where w_i and w_0 are the weights of the insoluble part and the initial weight of BC.

Dissolution of BC in an NaOH/urea solution

The solubility of BC in an NaOH/urea aqueous solution was examined with a similar procedure according to the dissolution of BC in an NaOH solution. The urea concentrations were varied from 1 to 6 wt %. To minimize moisture uptake, the solvents were prepared immediately before use.

Preparation of an RBC film

To develop an RBC film, 3 g of BC was dissolved in an NaOH/urea solution at a suitable composition obtained from the previous study, and this was followed by a freeze–thaw process. The casting solution was spread over a Teflon plate. The thickness of this solution was controlled at 3.3 mm by manual adjustment of the height of the casting blade. The thickness of the membrane was measured with a micrometer (Mitutoyo, Tokyo, Japan) at various parts of a particular film. The casting solution was coagulated in a 5 wt % $CaCl_2$ aqueous solution for 30 min and treated with a 1 wt % HCl solution for 10 min. After that, the membrane was washed with DI water until the drain water reached a pH of 7 and was air-dried at room temperature.

Preparation of a nanoporous structure

To avoid chemical contamination, supercritical CO_2 drying was applied in the preparation of the nanoporous structure. First, the RBC films were dipped in DI water for 24 h. After that, to replace the water with ethanol, the swollen films were immersed in 10, 30, 50, and 70 wt % ethanol for 30 min and finally immersed in 100 wt % ethanol for 1 h. Lastly, the swollen membranes were dried by supercritical CO_2 drying. In the drying procedure, the films were placed in a vessel inside a high-pressure cell with an inner diameter of 10 cm. The cell was immediately filled with supercritical CO_2 at 40°C and 1200 psi (critical point of carbon dioxide = 1072 psi, critical temperature = 31°C). The temperature and pressure were selected so that the CO_2 and ethanol inside the membrane were fully miscible. Subsequently, the cell was flushed by the addition of fresh CO_2 at the controlled temperature and pressure of 40°C and 1200 psi, respectively, for 2 h to replace the residual ethanol inside, and then the system was slowly

depressurized at a constant rate of 150 psi/min to remove the CO_2.

Characterization of the membranes

Elemental analysis of the membranes

The membranes were cut into particles and vacuum-dried for 24 h before the analysis of the elemental contents. The contents of nitrogen in the membranes were determined with an elemental analyzer (CHN-2000 analyzer, St. Joseph, MI). The contents of calcium and sodium were determined with an X-ray fluorescence (XRF) spectrometer (model ED2000, Oxford, United Kingdom).

Scanning electron microscopy (SEM)

SEM micrographs were taken with a scanning electron microscope (model JSM-5410LV, JOEL, Tokyo, Japan). The RBC film was frozen in liquid nitrogen, immediately snapped, and then vacuum-dried. The free surfaces were sputtered with gold and photographed. SEM was obtained at 15 kV, which was considered to be a suitable condition because too high an energy level could burn the samples.

Wide-angle X-ray diffractometry

X-ray diffraction was measured with an X-ray diffractometer (model D8 Discover, Bruker AXS, Karlsruhe, Germany). X-ray diffraction patterns were recorded with CuK_α, radiation (λ = 1.54 Å). The operating voltage and current were 40 kV and 40 mA, respectively. Samples were scanned from 10°–40° 2θ at a scan speed of 3°/min. The crystallinity index (CI) was calculated from the reflected intensity data with Segal et al.'s method:[22,23]

$$\mathrm{CI} = \frac{I_{020} - I_{am}}{I_{020}}$$

where I_{020} is the maximum intensity of the lattice diffraction and I_{am} is the intensity at 2θ = 18°.

Equilibrium water content (EWC)

EWC (or the water absorption percentage) was determined by the immersion of the dried membrane in DI water at room temperature until equilibration. After that, the membrane was removed from the water, and excess water at the surface of the membrane was blotted out with Kimwipes paper (Kimberly-Clark Corp., Ogdensburg, NY). The weight of the swollen membrane was measured, and the procedure was repeated until there was no further weight change. The water content was calculated with the following formula:

$$\mathrm{EWC}\ (\%) = \frac{W_h - W_d}{W_d} \times 100$$

where W_h and W_d denote the weights of the hydrate and dry membrane, respectively.

Tensile property testing

RBC films were cut into strip-shaped specimens 10 mm wide and 10 cm long. The maximum tensile strength and break strain of RBC films were determined with a Lloyd 2000R (Southampton, UK) universal testing machine. The test conditions followed ASTM D 882. The tensile strength and break strain were the average values determined from 10 specimens.

Brunauer–Emmett–Teller (BET) surface analysis

The pore size and surface area of the RBC membranes were determined with a BET surface area analyzer (model ASAP 2020, Micrometrics Corp., Atlanta, GA).

RESULTS AND DISCUSSION

Cellulose does not melt, but it undergoes thermal degradation at a high temperature.[24] However, cellulose can be converted into a liquid form by either direct dissolution or derivatization and subsequent dissolution of the derivative. The strong interchain and intrachain hydrogen bonding involved in the crystalline regions makes reactions and dissolution of cellulose difficult. There are a number of liquefaction methods available. However, these processes lead to problematic environmental loads.[25] In this study, BC was dissolved in a low-toxicity aqueous solution in a procedure similar to that developed by the research group at Wuhan University in China.[17] BC was placed in a solution of NaOH and urea at various compositions and stirred at room temperature until it was transformed into a highly swollen gel slurry. The slurry was frozen overnight and then thawed to obtain a clear solution. According to previous reports,[21,26,27] the freeze–thaw process disrupted hydrogen bonding between and within cellulose chains and increased interaction between the solvent and cellulose.

Dissolution in an NaOH aqueous solution

BC (3 wt %) was dissolved in an NaOH solution at concentrations varying from 1 to 10 wt %. As shown in Figure 1, the solubility of BC linearly increased

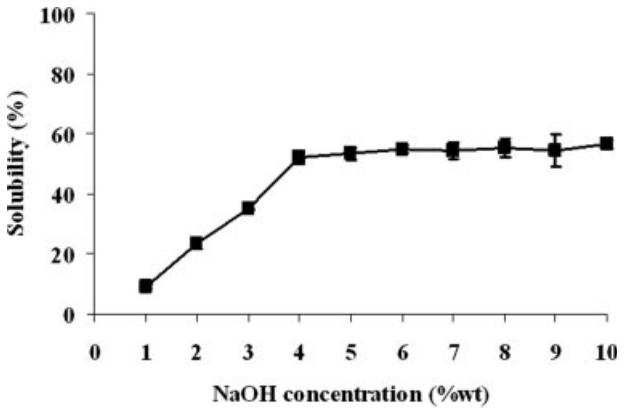

Figure 1 Effect of the NaOH concentration on the solubility of 3 wt % BC.

from 9.12 to 52.13% when the NaOH concentration increased from 1 to 4 wt %. However, the solubility slightly increased from 52.13 to 56.43% when the NaOH concentration was increased from 4 to 10 wt %. An increase in the NaOH concentration of more than 4 wt % hardly affected the BC solubility. Therefore, the optimum NaOH concentration for BC dissolution was 4 wt %. For the dissolution of cellulose from plants, Zhou and Zhang[15] reported an optimum NaOH concentration of 6 wt %. The optimum conditions involved swelling cellulose in NaOH followed by the freeze–thaw process.

Dissolution in an NaOH/urea aqueous solution

Figure 2 shows the results for the solubility of BC in the presence of various urea concentrations in a 4 wt % NaOH solution. The solubility of BC dramatically increased from 77.67 to 92.33% when the urea concentration increased from 1 to 3 wt %. Nevertheless, the solubility only slightly increased from 92.33 to 93.21% as the urea concentration varied from 3 to 8 wt %. The addition of 3 wt % urea to a 4 wt % NaOH solution significantly enhanced the solubility of 3 wt % BC from 52.13 to 92.33%. As a result, the optimum composition of the NaOH/urea solution for the dissolution of BC was 4 wt % NaOH and 3 wt % urea.

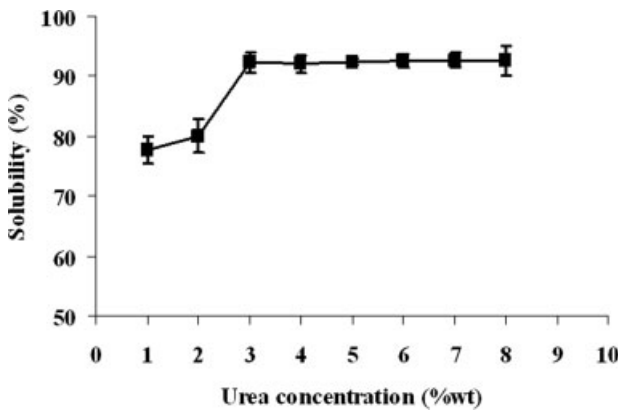

Figure 2 Effect of the urea concentration on the solubility of 3 wt % BC in 4 wt % NaOH.

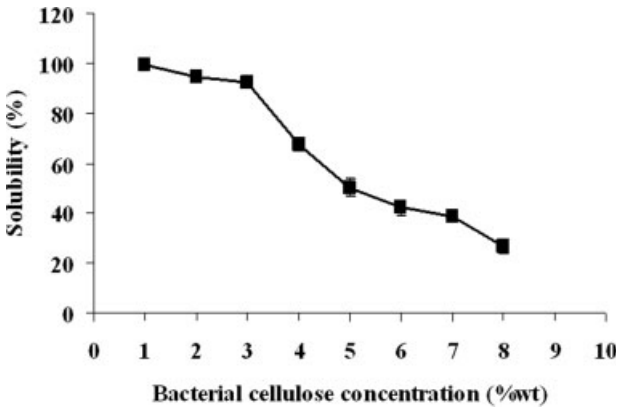

Figure 3 Effect of the cellulose concentration on the solubility of BC in a 4 wt % NaOH/3 wt % urea aqueous solution.

The enhancement of cellulose solubility in an NaOH aqueous solution by the addition of urea was previously reported.[12,13,15,17] The addition of 2–4 wt % urea significantly improved the solubility of plant cellulose in a 6–8 wt % NaOH solution, and the optimum composition was 6 wt % NaOH/4 wt % urea.[12] It was previously found that NaOH/urea aqueous solutions as nonderivatizing solvents break the intramolecular and intermolecular hydrogen bonding of cellulose and prevent the approach toward other cellulose molecules, leading to the good dispersion of cellulose to form an actual solution.[28]

Solubility of BC in an NaOH/urea aqueous solution

Figure 3 shows the solubility of BC in a 4 wt % NaOH/3 wt % urea aqueous solution. The solubility of BC slightly decreased from 99.12 to 92.33% as the amount of BC was increased from 1 to 3 wt %. Nonetheless, the solubility of BC dramatically decreased from 92.33 to 26.34% when the proportion of

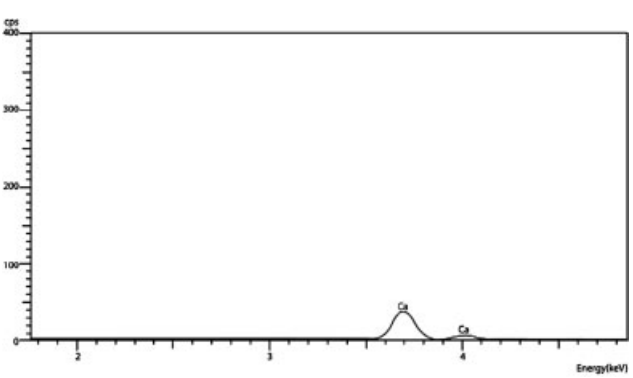

Figure 4 XRF spectrum of an RBC film.

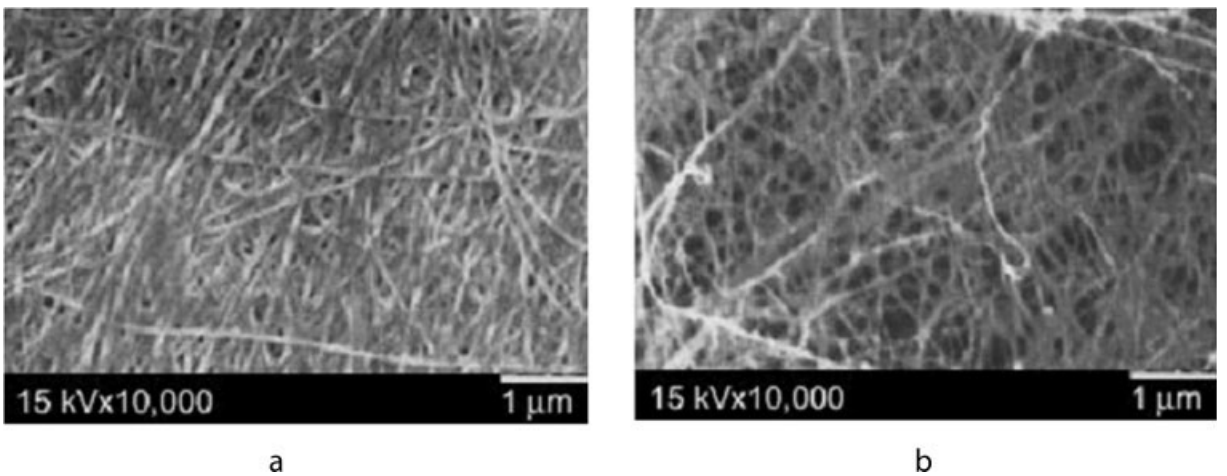

Figure 5 SEM images of the surface morphology of a BC film (a) before and (b) after reswelling and supercritical CO_2 drying.

BC was increased from 3 to 8 wt %. To obtain BC solubility of more than 90%, the amount of BC in the solution should not be greater than 3 wt %. The poor solubility of hydrogel cellulose in NaOH was previously reported.[29] The formation of a gel polymer in a solution is likely to cause the reduction of the free volume of the solvent. In this study, the solubility of a BC gel in an NaOH/urea solution was found to be limited around 3 wt %. For membrane fabrication, the cellulose concentration should be high enough to form a membrane with an integral structure.[30] As a result, 3 wt % BC in a 4 wt % NaOH/3 wt % urea aqueous solution was the optimal composition to be used for the preparation of a casting solution for the RBC film formation. Subsequently, the gel solution was coagulated in a 5 wt % $CaCl_2$ solution and treated with a 1 wt % HCl solution.

Analysis of the elemental contents

Elemental analysis revealed the absence of nitrogen in the fabricated film. Therefore, urea was completely removed from the film during coagulation and washing. The XRF spectroscopy results indicated that the calcium concentration in the membrane was 623 ppm, and no sodium peak was observed (Fig. 4). Therefore, NaCl and NaOH were totally removed from the developed film. The result was similar to that of a regenerated cellulose film from cotton in an NaOH/urea aqueous solution prepared by Zhou et al.[13] In that report, the nitrogen element content in the film was less than 0.1%, and the calcium and sodium contents in the film were nearly zero.

Characteristics of the RBC membrane

The physical properties of the RBC film were characterized and compared to those of the biosynthesized BC film from our previous work.[5] SEM micrographs of the BC and RBC films are compared in Figures 5 and 6. Both BC and RBC films displayed an ultrafine fiber network structure of fibrils with an average

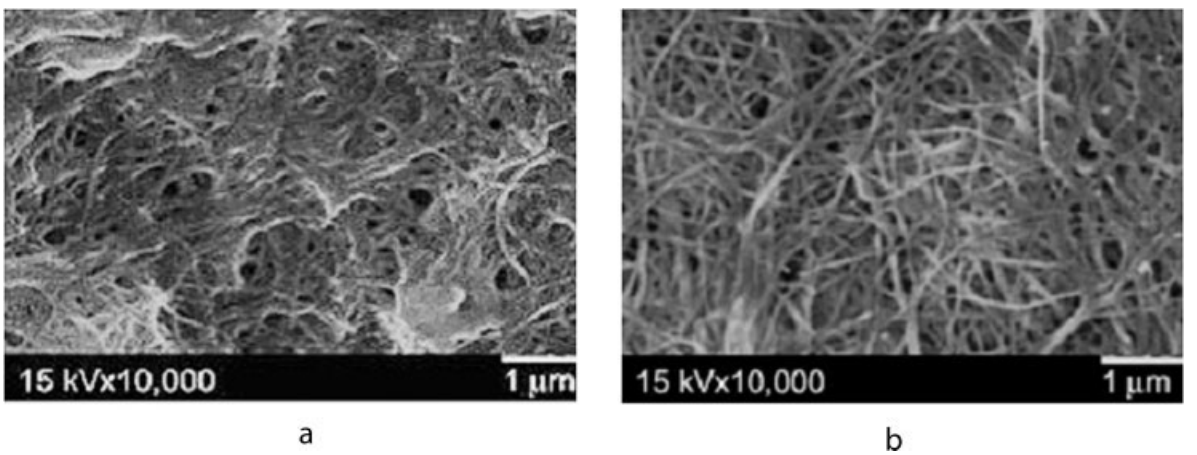

Figure 6 SEM images of the surface morphology of an RBC film (a) before and (b) after reswelling and supercritical CO_2 drying.

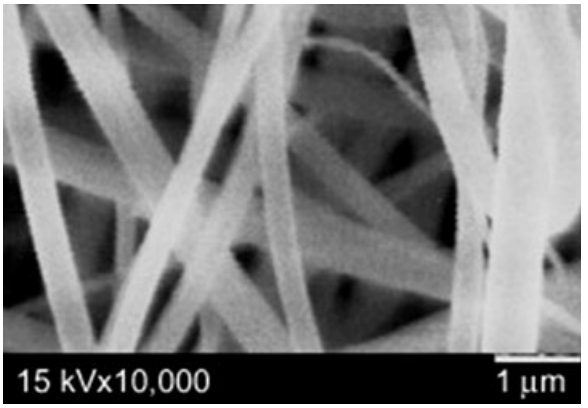

Figure 7 SEM of the surface of a commercial-grade regenerated cellulose membrane from plants (Whatman membrane filter).

diameter of 0.05 µm. Because BC was synthesized extracellularly into nanosized fibrils by the bacteria, the RBC membrane had a nanofiber network structure, whereas a sample of regenerated plant cellulose (Whatman filters, catalog no. 1822070 Whatman International Ltd., Maidstone, UK) had a microfiber network structure of fibrils with an average diameter of 0.5 µm, as demonstrated in Figure 7.

Pore size distributions of the dried BC and RBC films are shown in Figure 8. The average pore diameter and surface area of the BC films in the dry form, determined by BET, were 224 Å and 12.62 m^2/g, respectively.[5] However, after BC was reswollen in water at 30°C and dried with the supercritical CO_2 drying method, the pore was enhanced to 0.2–1.0 µm. The structure of the RBC film was much denser with a pore diameter of 2–10 Å. The average pore diameter of the RBC film after swelling in water at 30°C followed by supercritical CO_2 drying was 12.63 Å

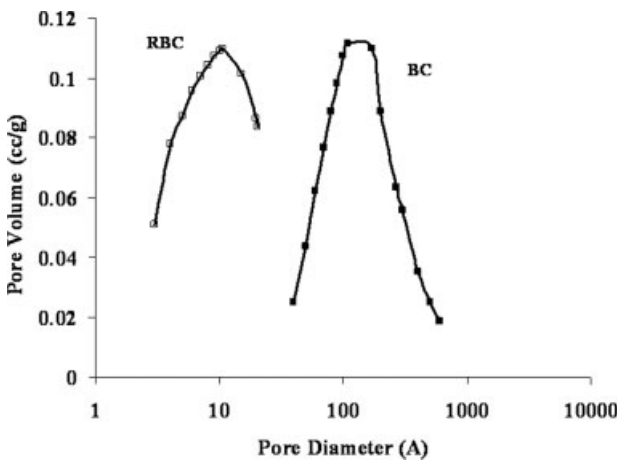

Figure 8 Pore size distributions of dried BC and RBC films (after reswelling and supercritical CO_2 drying) from a desorption pore volume plot by BET.

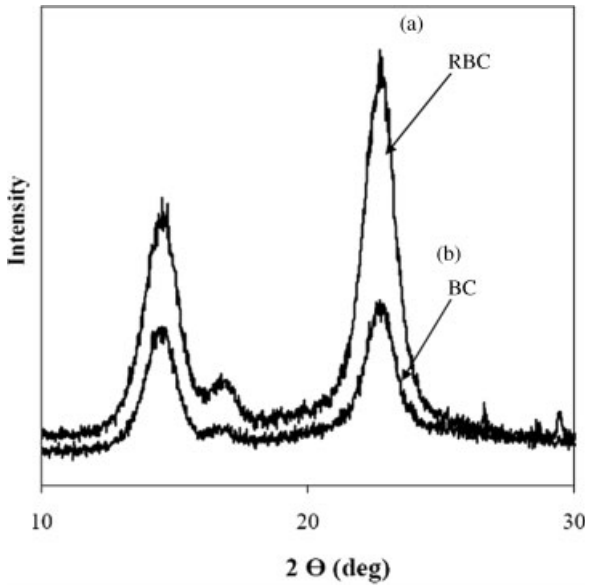

Figure 9 X-ray diffraction patterns of (a) RBC and (b) BC films.

with a 17.57 m^2/g surface area. Moreover, although the BC film demonstrated a remarkable capacity to hold water, the water absorption capacity of the RBC film dropped incredibly to 49.7%, which was only 1/10 of that of the BC film. This could imply that the hydrogen bonds in RBC might form a more tightly packed network than natural BC. Therefore, water could penetrate the film less.

The X-ray diffraction patterns of BC and RBC films in the range of 2θ = 10–30 are shown in Figure 9. The CI, reflective-angle, and *d*-spacing values of the BC and RBC membranes are shown in Table I. The reflective angle and *d*-spacing of the BC and RBC films matched those obtained from typical BC cultured in static circumstances.[23,31] Peaks at 2θ values of 14.6, 16.9, and 22.7° correspond to $(1\bar{1}0)$, (110), and (200) planes of the cellulose I crystalline form.[32] Similarities of the diffraction patterns were observed for both BC and RBC films; however, the peak intensity of the RBC film was much higher because of the denser structure of the RBC membrane. The CI value of RBC (83.6%) was slightly higher than that of BC (75.6%), suggesting a higher level of hydrogen bonding between the cellulose chains of RBC. The CI of the BC film in this work was slightly lower than that in the previous report.[23] The differences in the

TABLE I
CI, Reflective-Angle, and *d*-Spacing Values of the BC and RBC Membranes

Membrane	CI	2θ [$d(1\bar{1}0)$]	2θ [$d(110)$]	2θ [$d(020)$]
BC	78.6	14.56° (6.08)	16.97° (5.22)	22.75° (3.91)
RBC	83.6	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)

obtained CI values might arise from the difference in the *A. xylinus* strain and the procedure and media for BC biosynthesis. In comparison, the regenerated cellulose membranes prepared from cotton linter in an NaOH/urea aqueous solution exhibited a relatively large pore size of 200–2000 nm (2rSEM, the average apparent pore size from scanning electron micrographs) and displayed the typical cellulose II crystal form with a CI value of 62–63%.[13] Therefore, besides the variation in the preparation procedures, the source of the cellulose strongly impacted the characteristics of the regenerated cellulose films.

From the mechanical analysis of the dried film with a thickness of 0.1 mm, the average tensile strength of RBC (4.32 MPa) was slightly less than that of BC (5.21 MPa). However, the regeneration process used in this study could improve the elongation behavior of the cellulose film. The break strain of RBC increased to 35.20% or 9.4 times that of the BC film (3.75%). This might be due to the changes in the membrane structure. The structure of the RBC network and the interaction between molecules formed by coagulation with a $CaCl_2$ solution differed from those of the BC film formed by bacterial synthesis. A small amount of calcium detected in the RBC membrane could be from the formation of calcium bridges by coagulation with a $CaCl_2$ solution as previously suggested for gel network formation by crosslinking with Ca^{2+} ions.[14] The RBC film structure was much denser, and the RBC fibrils curved instead of being almost straight as the BC fibrils. Moreover, the molecular weight of the polymer chains of RBC should be lower than that of BC as a result of the cutting and vigorous blending during the preparation of the RBC solution. In a previous study of chitosan membrane formation, with molecular weights varying from 190-310 kD to greater than 310 kD,[33] the dried films exhibited a greater break strain but a lesser break stress when the molecular weight decreased. For the solubility test, neither the BC film nor the RBC film dissolved in water or ethanol. Table II gives a summary of the RBC film characteristics in comparison with those of the BC film.

TABLE II
Characteristics of the Biosynthesized BC Film and the RBC Film Prepared from an NaOH/Urea Aqueous Solution with a Film Thickness of 0.1 mm

Property	BC film	RBC film
Tensile strength (MPa)	5.21 ± 0.82	4.32 ± 0.73
Break strain (%)	3.75 ± 0.50	35.20 ± 3.66
Young's modulus (MPa)	162.50 ± 10.94	14.35 ± 1.51
Pore diameter (Å)	224	2–10
Water absorption capacity (%)	509.0 ± 11.5	49.7 ± 4.2
Dissolution in water	No	No
Dissolution in ethanol	No	No

CONCLUSIONS

A novel nanostructure membrane was synthesized from BC in an NaOH/urea aqueous solution. BC (3 wt %) was dispersed in a 4 wt % NaOH/3 wt % urea solution, and this was followed by a freeze–thaw process to obtain a clear, dissolved BC solution. The casting solution was then coagulated in a 5 wt % $CaCl_2$ solution for 30 min and treated with a 1 wt % HCl solution. The developed film displayed a very dense porous structure with high mechanical strength. The tensile strength, elongation at break, and water absorption percentage of the RBC membrane with a thickness of 0.1 mm were 4.32 MPa, 35.20, and 50%, respectively. The tensile strength of the RBC film was comparable to that of the BC film, but the break strain was enhanced 9.4-fold. On the other hand, the water absorption capacity was reduced to 1/10. After the supercritical CO_2 drying, the formation of a nanoporous structure of RBC with an average pore diameter of 12.63 Å and a 17.57 m^2/g surface area was obtained. The membrane was water-resistant and did not dissolve in ethanol. Because the developed RBC membrane had a nanoporous structure and was chemically stable and biodegradable and its preparation was environmentally friendly as well, the film has potential uses in several nanofiltration applications.

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