Biosynthesis and Characterization of Bacteria Cellulose–Alginate Film

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ABSTRACT: A novel polysaccharide membrane containing alginate in bacterial cellulose matrix was synthesized by *Acetobacter xylinum* under static conditions using a culture medium supplementation with sodium alginate. By increasing alginate content, the bacterial cellulose–alginate (BCA) membrane was more hydrophilic and the film structure became denser with the smaller average pore size. Scanning electron microscope images displayed the deposits of alginate gel on the surfaces of the multilayer cellulose film. The declines in the tensile strength, the Young's modulus, and the elongation at break of the BCA membrane were dependent on the degree of alginate supplement. The BCA membrane showed higher water absorption capacity. The addition of alginate slightly affected the water vapor transmission rate but remarkably decreased the oxygen transmission rate of the membrane. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 1581–1588, 2010

Key words: bacteria cellulose; alginate; *Acetobacter xylinum*; characterization; film

INTRODUCTION

Cellulose is the most abundant natural polysaccharide produced by plants and microorganisms. It was formed out of glucose-based repeat units, connected by 1,4-β-glucosidic linkages.[1] Cellulose fibrils are highly inelastic and insoluble.[2] The β-1,4 linkage results in a ribbon-like molecule, which is suited for forming fibril via hydrogen bonding.[3] The bacterial cellulose (BC) film with its highly pure nanofibril cellulose network can be formed by the *Acetobacter species*. It is distinguished by its mechanical strength, water absorption capacity (WAC), and crystallinity. Its unique structural features and properties facilitate diverse applications, ranging from food matrix, dietary fiber, wound-dressing, carrier for mammalian cell culture, immobilization of enzymes, and other biomolecules, as well as diaphragms in speakers for acoustic and separation membranes.[4–8] The pellicle which is flat can be easily processed into a uniform porous membrane, whereas plant cellulose is often interspersed with lignin, hemicellulose, and pectin leading to nonuniformity in porosity and inconsistent permeability. Furthermore, the production yield and the structure of BC can be changed from the control conditions such as composition of the culture media, pH, temperature, and oxygen tension as well as drying procedure.[1,9] The specific application of BC as a dialysis membrane was examined by Shibazaki et al. (1993).[10] The BC film showed a significantly higher permeation rate and a greater molecular weight cut-off when compared with regenerated cellulose membrane. The BC membrane was investigated for the pervaporation of aqueous-organic mixtures.[4,11] It was found that the permeate flux was incredibly high but the selectivity was fairly low.

Among the hydrophilic polysaccharide membranes, alginate film has gained special interest for its high flux and separation factor.[12–15] Alginate is naturally polysaccharide of linear copolymers of (1–4)-linked β-D mannuronic acid and α-L-guluronic acid, derived primarily from brown seaweed. Alginate can be transformed into the most widely used water-soluble form, sodium alginate, through the incorporation of sodium salt. However, alginate membrane has poor stability in aqueous solutions because of its highly hydrophilic character due to both of its carboxyl and hydroxyl groups, resulting in significant reduction of membrane selectivity and mechanical strength.[15]

The present research aimed to develop a composite film of bacterial cellulose–alginate for the application in membrane separation. To increase the hydrophilic property and generate the dense film structure, the biosynthesis of bacterial cellulose–alginate film was developed by the supplement of sodium alginate into the medium under static

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cultivation of *Acetobacter xylimun.* For further application in membrane systems, the effects of alginate content on the membrane properties, for instance, its structure, pore morphology, chemical structure, mechanical strength, WAC, and permeability of water vapor and oxygen were investigated.

MATERIALS AND METHODS

Culture, culture media, and method

The *Acetobacter xylinum* AGR 60 was kindly supplied by the laboratory of Pramote Tammarate (the Institute of Food Research and Product Development, Kasetsart University, Bangkok). The medium for the inoculums was coconut-water containing 5.0% sucrose, 0.5% ammonium sulfate $(NH_4)_2SO_4$, and 1.0% acetic acid. The medium was sterilized at 121°C for 15 min. Precultures were prepared by transferring 50 mL of a stock culture to 1000 mL of medium in 1500 mL bottles and incubated statically at 30°C for 5 days. After the surface pellicle was removed, the 5% (v/v) preculture broth was added to the main culture medium supplementation with different sodium alginate content. The 75 mL of activated medium was inoculated in a pretidish and kept at 30°C for 7 days. The developed gel-like cellulose pellicle was first purified by washing with deionized (DI) water and then was treated with 1% (w/v) NaOH at 35°C for 24 h to remove bacterial cells and rinsed with DI water until the pH was 7. Afterward, the purified sheets were air-dried at room temperature) (30°C) and stored in plastic film before use.

Characterization of membranes

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of the membranes were measured at wave numbers ranging from 2000 cm^{-1} to 800 cm^{-1} at a resolution of 4 cm^{-1} with a Nicolet (US) SX-170 FTIR spectrometer.

Water absorption capacity

To determine the WAC, the dried membranes were immersed in DI water at room temperature) (30°C) until equilibration. After that the membranes were removed from the water and excess water at the surface of the membranes was blotted out with Kimwipes paper. The weights of the swollen membranes were measured, and the procedure was repeated until no further weight change was observed. The water content was calculated with the following formula:

$$\text{WAC } (\%) = \frac{W_h - W_d}{W_d} \times 100$$

where W_h and W_d denoted the weight of hydrate and dry membrane, respectively.

Mechanical strength

The air-dried membranes were cut into strip-shaped specimens 10 mm width and 10 cm long. The maximum tensile strength, Young's modulus, and break strain of BC and BCA films were determined with a Lloyd (Southampton, UK) 2000R universal testing machine. The test conditions followed ASTM D 882. The tensile strength, Young's modulus, and break strain were the average values determined from 10 specimens.

Scanning electron microscopy

The films were frozen in liquid nitrogen, immediately snapped, vacuum-dried, and then sputtered with gold and photographed. Images were taken on a JOEL (Tokyo, Japan) JSM-5410LV scanning electron microscope (SEM). The accelerating voltage was adjusted to 15 kV. The specimen was examined at magnifications from 2000× to 10,000×.

Brunauer-Emmett-Teller (BET) surface analysis

The pore size and surface area of the membranes were determined with a BET surface area analyzer. To remove moisture from the film samples, the samples were placed in sample cells, which were then heated upto 348 K for 5 h and cooled down to room temperature before the BET analysis. The BET pore size and surface area were determined with N_2 adsorption at 77 K in a Micromeritics (Atlanta, GA) ASAP 2020.

Water vapor permeability measurement

The water vapor transmission rate (WVTR) of the dry membranes with area of 50 cm^2 was determined with a Lyssy (Zollikon, Switzerland) L80-4000 water vapor permeation tester. The test conditions followed ISO 15106-1. The determination of WVTR was done at 38°C and 90% relative humidity. One side of the membrane was exposed to a humid air atmosphere and the other side was exposed to a nitrogen atmosphere. As water solubilized into the membrane and permeated through the sample film, nitrogen gas swept and transported the transmitted water vapor molecules to a calibrated infrared sensor. The response was reported as a transmission rate.

The oxygen permeability measurement

Oxygen transmission rate (OTR) of the dry membranes was determined with a oxygen permeation

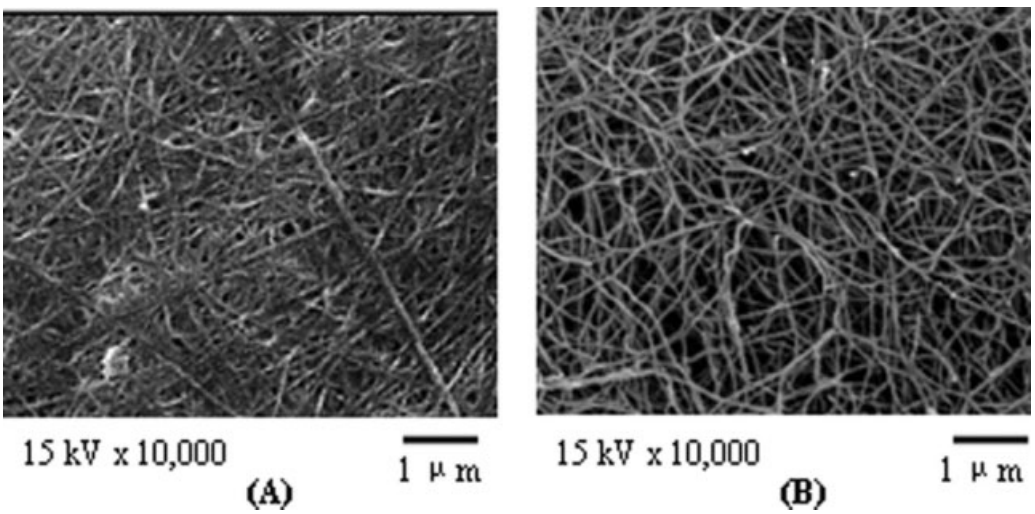

Figure 1 SEM images of surface morphology of BC film in: (A) dry form and (B) reswollen form.

analyzer: Illinois Instruments (Johnsburg, IL) Model 8000. The test conditions followed ASTM D3985. The determination of OTR was done at 23°C and 0% relative humidity. The membrane was held in such a manner that it separated two side of a test chamber. One side was exposed to an oxygen atmosphere and the other side was exposed to a nitrogen atmosphere. Testing was completed when the concentration of oxygen in the nitrogen side was constant.

RESULTS AND DISCUSSION

In case of cultivation in a shake flask or in a stirred-tank reactor, the addition of 0.04% (w/v) sodium alginate into culture medium enhanced yields and changed the morphology of cellulose.[16] Our preliminary test demonstrated that under static cultivation, to avoid the inhibition of cell and film production, the maximum amount of sodium alginate addition in the culture medium was limited at 1.0% (w/v) (data not shown). Therefore, a study of the alginate supplement in culture medium was performed in the concentration range of 0–1.0% (w/v). The structure, pore morphology, mechanical strength, chemical structure, WAC, as well as well as WVTR and OTR of the developed films were then examined to investigate the effect of alginate supplement.

Membrane morphology

The SEM images for surface morphology of BC and BCA membranes are shown in Figures 1 and 2, whereas the SEM images for the cross section are shown in Figures 3 and 4. Regarding the definitions, the BC and BCA membranes refer to the BC

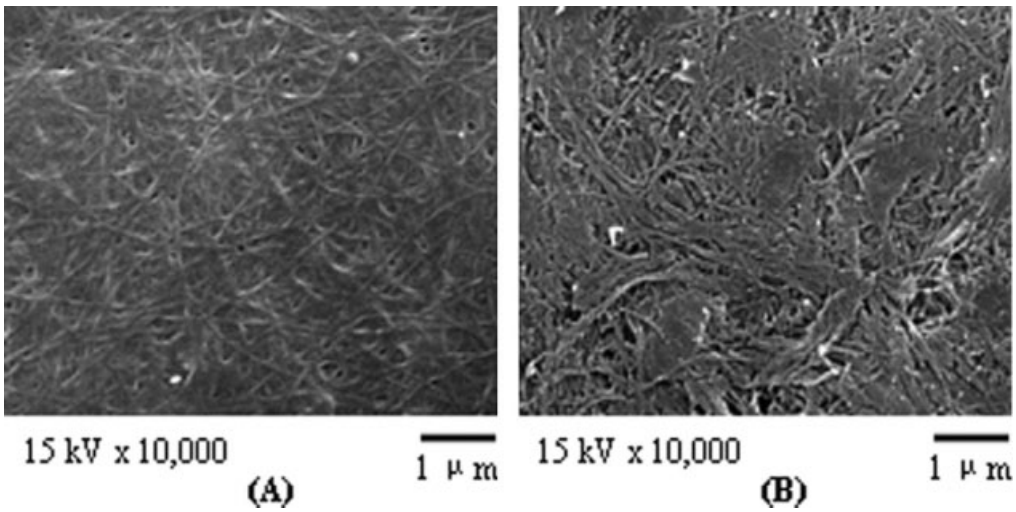

Figure 2 SEM images of surface morphology of 1% Al-BCA film in: (A) dry form and (B) reswollen form.

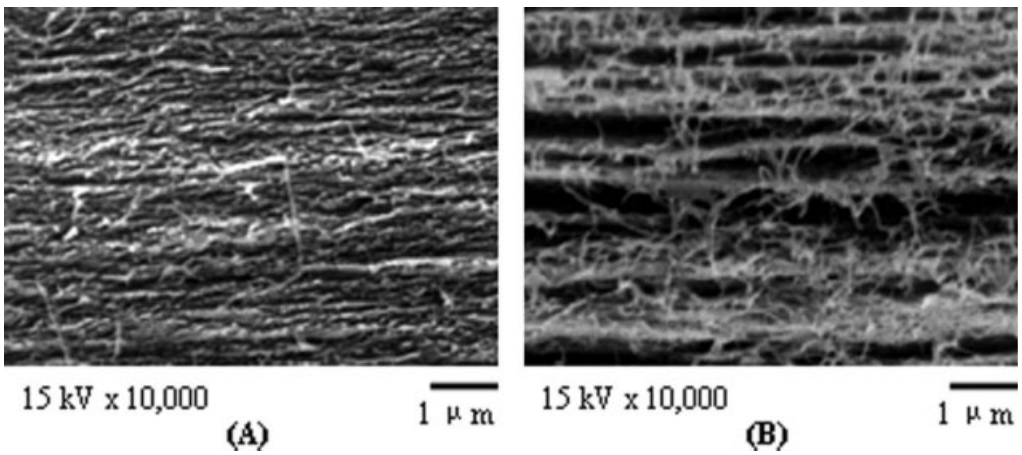

Figure 3 SEM images of cross section of BC film in: (A) dry form and (B) reswollen form.

membranes without and with the addition of alginate in culture medium, respectively, whereas, *x*% Al-BCA refers to the BCA membrane with *x*% (w/v) sodium alginate supplement in culture medium. The SEM images of surface morphology present the porous film composed of a continuous network of cellulose nanofibrils of 50–100 nm. According to high WAC of the membranes, the reswollen membranes exhibited a looser structure and contained larger pores than those of the dried ones. From the surface morphology images of the BCA membrane (Fig. 2), with the supplement of sodium alginate into the culture medium, the deposits of alginate gel on the film surfaces were observed and the apparent pore sizes of BCA films decreased with increasing the percent of alginate. Since alginate gel was well incorporated into the cellulose fibril network and filled the pores, the structure of the BCA membrane was denser with the smaller pore size than that of the BC membrane. As shown from the cross section images of BC and BCA membranes (Figs. 3 and 4), the membranes possessed numerous sheets composed of a network of nanofibrils. The membranes in dry form were made of thin sheets that were tightly packed, whereas the reswollen ones had looser packed structures.

FTIR analysis

FTIR spectroscopy is utilized in determining the specific functional groups or chemical bonds in a material. The FTIR spectra of the BC and BCA membranes were measured with the wave number ranging from 2000 cm^{-1} to 800 cm^{-1} as shown in Figure 5. All the characteristic bands of the BC film were present in the spectra of the BCA films without any occurrence of new peaks. The BC membrane showed a band at 1647.0 cm^{-1}, which was attributed to the glucose carbonyl of the cellulose. The carbonyl group band for BCA films slightly shifted from 1647.0 cm^{-1} to lower wave numbers, 1645.4–1646.6 cm^{-1} and the band became broader, which might indicate some weak physical interactions between the carbonyl groups of cellulose and sodium alginate. No evidence of peak shift that would indicate a chemical interaction. However,

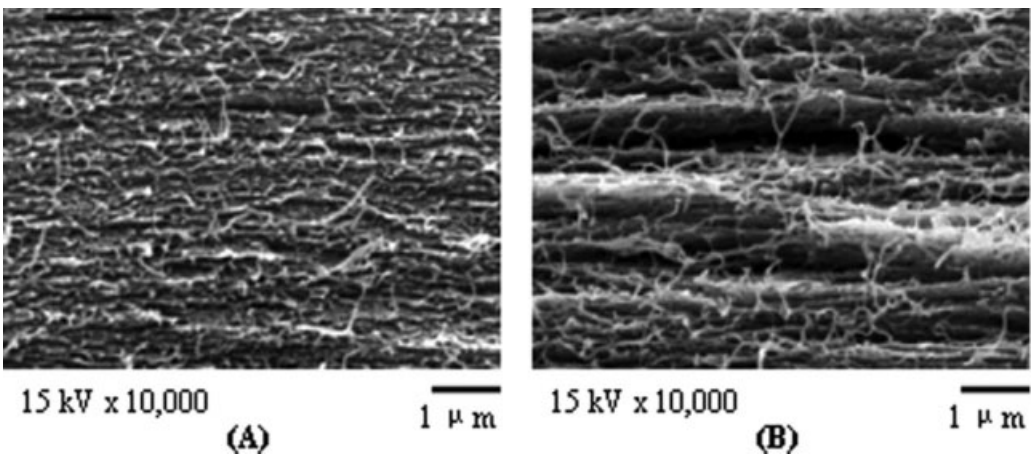

Figure 4 SEM images of cross section of 1% Al-BCA film in: (A) dry form and (B) reswollen form.

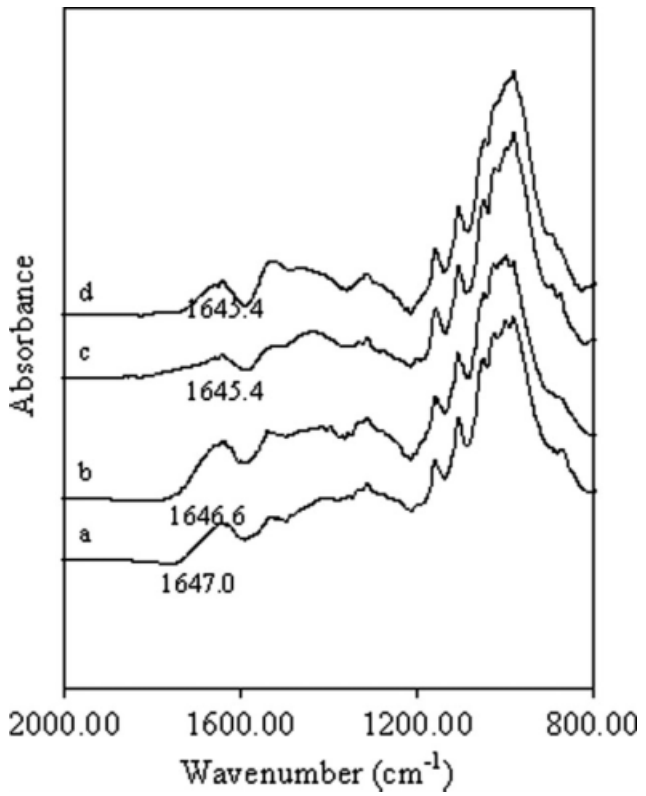

Figure 5 The FTIR spectra of the BC and BCA membranes in wave numbers ranging from 2000 cm^{-1} to 800 cm^{-1}: (a) BC; (b) 0.5% Al-BCA; (c) 0.75% Al-BCA; and (d) 1.0% Al-BCA.

under agitated culture, Zhou et al.[16] reported the interaction from the shift of —OH and C—O—C bands in the presence of sodium alginate in the culture medium. From our previous work on the alginate–bacteria cellulose blend membrane,[17] changes in the region of 1640–1600 cm^{-1} were reported, which indicated some interactions between the hydroxyl group of cellulose and the carboxyl group of alginate. As the membrane composition and structure were different, the interaction of alginate and cellulose in the BCA membrane might not be the same as that of the blend membranes.[16,17]

Mechanical properties

End-use applications usually involve some degree of mechanical loading, therefore, the effects of alginate content on the mechanical properties such as the tensile strength, Young's modulus, and elongation at break were examined. As shown in Figure 6, the tensile strength of the BC membrane at the average thickness of 40 μm was 5.30 MPa, whereas those of the 0.5–1.0% Al-BCA membranes varied from 4.71 MPa to 3.80 MPa. The decreasing of the tensile strength of the BCA membranes was in a linear manner ($R^2 = 0.9854$) with the amount of alginate content.

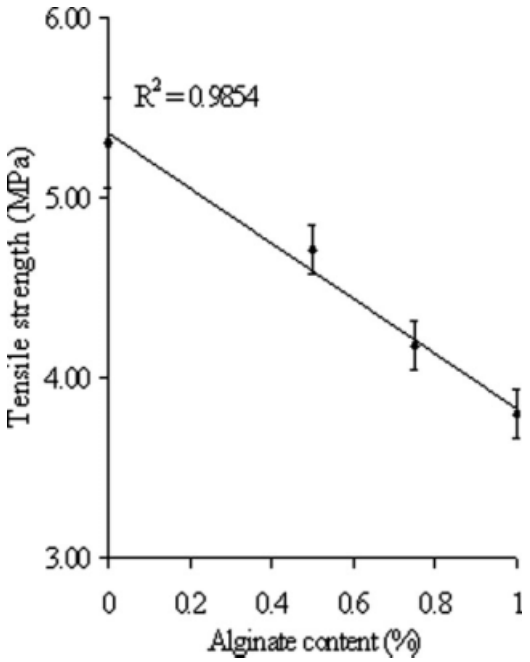

Figure 6 The tensile strength of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

The decrease of Young's modulus of the BCA membranes as a function of alginate content was similar to that of the tensile strength (Fig. 7). The Young's modulus of the BC and the 1.0% Al-BCA membranes were 172.8 MPa and 144.4 MPa, respectively. The elongation at break also decreased when

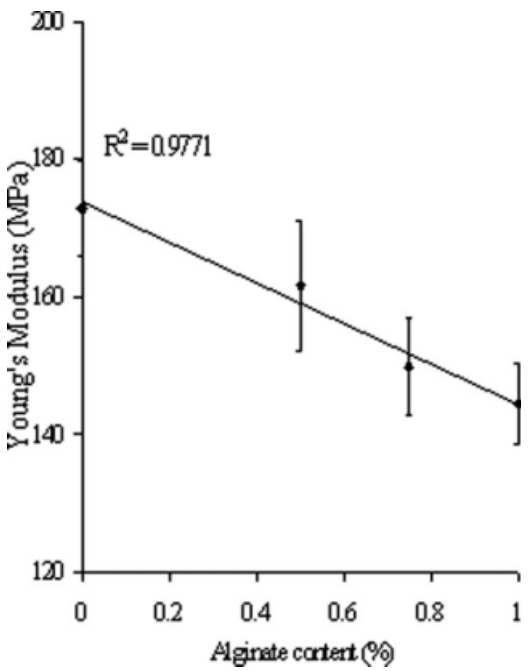

Figure 7 The Young's modulus of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

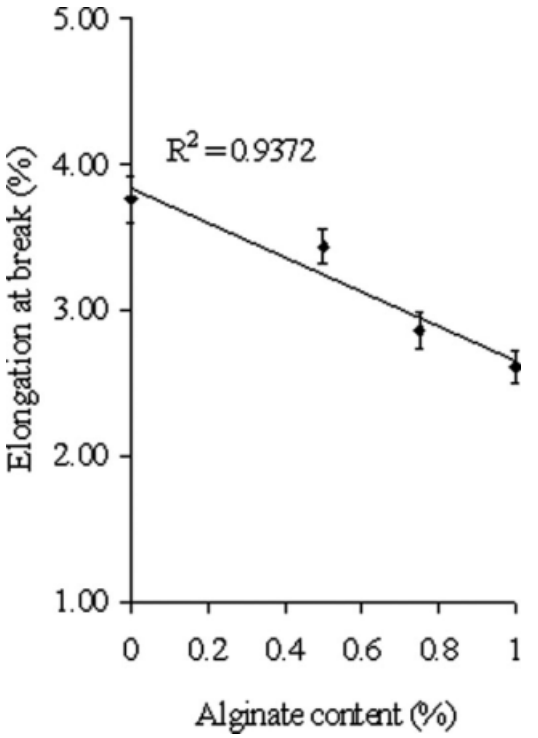

Figure 8 The elongation at break of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

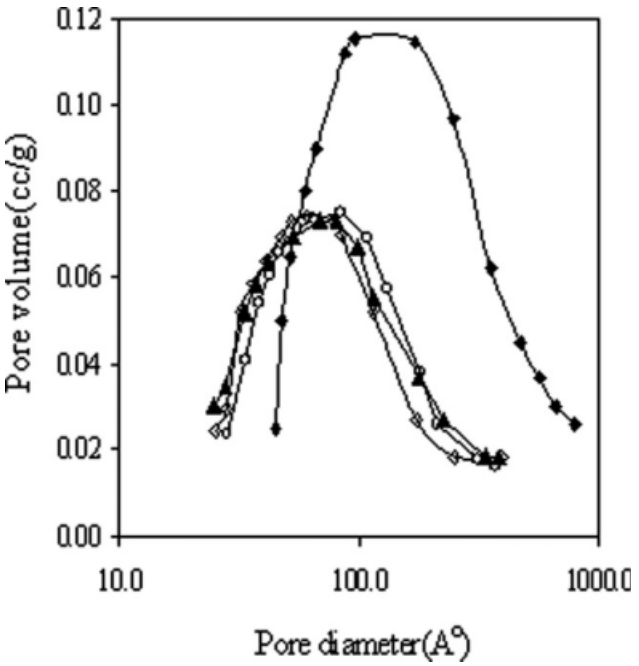

Figure 9 The pore size distribution of the BC and BCA membranes: (a) BC [◆]; (b) 0.5% Al-BCA [○]; (c) 0.75% Al-BCA [▲]; and (d) 1.0% Al-BCA [□].

increasing the alginate content as shown in Figure 8. The elongation at break of the BC and the 1.0% Al-BCA membranes were 3.76% and 2.61%, respectively.

With the supplement of alginate, alginate was incorporated into the bulk of cellulose fibril networks of the BCA membrane. A comparison of mechanical properties of the BCA film with 0.5–1.0% Al and the BC film indicated statistically significant decreases for tensile strength ($P < 0.005$), elongation at break ($P < 0.005$), and Young's modulus ($P < 0.05$) for a one-tailed test. The effect of alginate content on the mechanical properties of the BCA membranes was similar to those previously observed in the blend membranes of BC and alginate blend in NaOH/urea,[17] cellulose cuoxam with alginate,[18] and cotton cellulose and alginate blend in NaOH/urea aqueous solution.[19] Although the cellulose/alginate blend membranes were mechanically weaker than cellulose membranes, they were mechanically stronger than the alginate membranes with a promising performance for pervaporation dehydration.[20] The sodium alginate film was mechanically weak and had poor stability in aqueous solution.[15] The presence of alginate in the cellulose–alginate composite could enhance the molecular motion of cellulose in the blend and perturbed the strong hydrogen bond of pure cellulose resulting in the reduction in mechanical strengths.[17–19]

Porosity

The results of the pore size distribution of the BC and BCA membranes from BET analysis are shown in Figure 9. Corresponding to the results from the SEM images, the BCA membranes had an average pore size much smaller than that of the BC membrane and the average pore diameter decreased with increasing alginate content, whereas the surface area slightly decreased (Table I). A possible explanation was that by means of adding sodium alginate in the culture medium, as in this work, the alginate gel deposited on the film sheet, the alginate molecules diffused through the pores and partially filled the pores of the films. The SEM observations of cross sections of the reswollen BC and BCA membranes (Figs. 3 and 4) revealed that the film sheets united together to form the bulk membrane. Therefore, the presence of alginate incorporated into the cellulose fibril networks and filled pores resulted in a significant reduction of the membrane pore size. From our

TABLE I
Surface Areas and Pore Diameters of the Dry BC and BCA Membranes Analyzed by BET Analyze

Alginate content (% w/v)	Average pore diameter (Å)	Surface area (m^2/g)
0	224	12.6
0.5	97	12.1
0.75	64	11.8
1	39	11.2

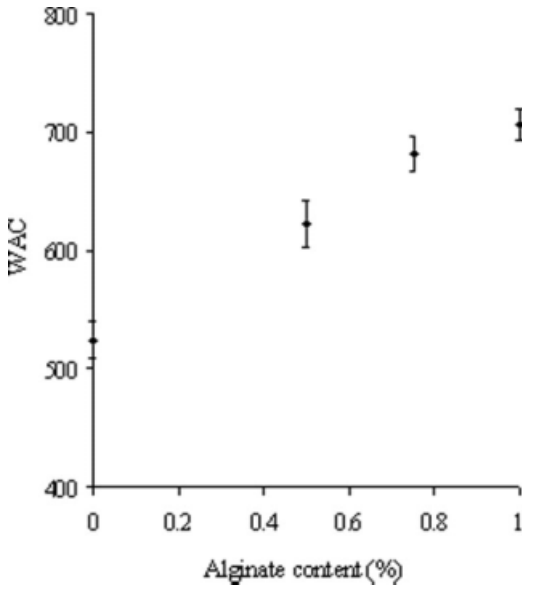

Figure 10 The water absorption capacity (WAC) of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

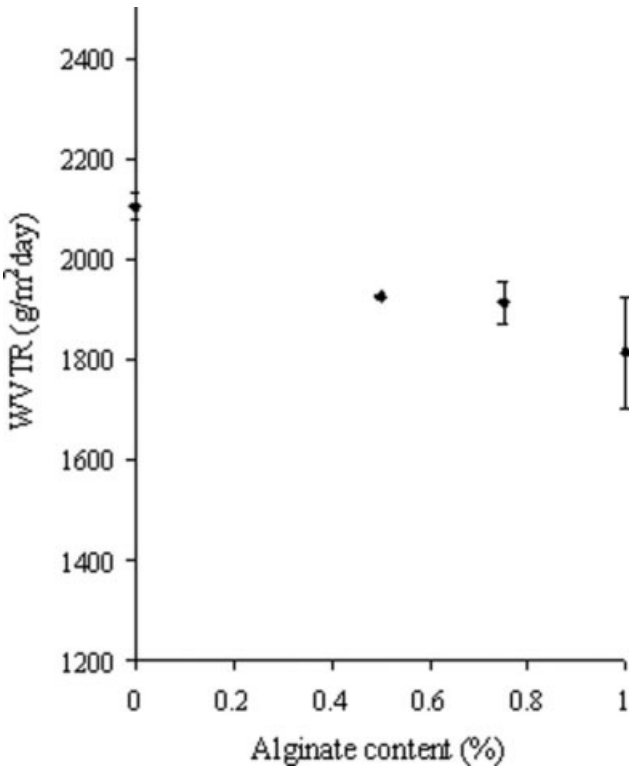

Figure 11 The water vapor transmission rate (WVTR) of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

previous observation in the alginate–bacteria cellulose blend membrane, the apparent pore size of the blend membrane to some extent also decreased with the increase in the alginate content.[17]

Water absorption capacity

From Figure 10, the WAC of the BC and the 1.0% Al-BCA membranes were 542% and 706%, respectively. The WAC increased with the alginate content. Alginate is very hydrophilic; the water molecule is easily absorbed into the alginate membrane.[14] As alginate was incorporated into the BCA membrane, the film was more hydrophilic. Therefore, the BCA film was enabled to adsorb and retain more water than the BC film.

Water vapor permeability test (WVTR)

The BC and BCA films are highly hydrophilic. During the WVTR analysis, a feed of water vapor (90% relative humidity) could make the membranes in a swollen state. The solubilization of water into the membrane has a strong influence on the morphology and porous structure of the membrane. A major change of porosity and pore size in form of the reswollen film was previously demonstrated (Figs. 1–4). Figure 11 demonstrates the water vapor permeability of the films at an average thickness of 40 μm. The water vapor transmission rate (WVTR) slightly decreased when increasing alginate content. Although the hydrophilic property and the WAC of the BCA membranes were improved, as a result of the denser structure and decrease in pore size from the supplement of alginate, the WVTR decreased relatively. In comparison with the BC film, the reduction in the WVTR of the 0.5, 0.75, and 1.0% Al-BCA membranes were at 8.5%, 9.1%, and 13.8 %, respectively. Compared with the WVTR of the BC membrane, the supplement of alginate (0.5–1.0%) causes a statistically significant decrease in WVTR calculated using a one-tailed *t*-test for a 95% confidence limit ($P < 0.05$).

Oxygen permeability test (OTR)

Figure 12 shows the oxygen transmission rate (OTR) of the membranes at an average thickness of 40 μm. As alginate content increased, the OTR decreased considerably due to the denser structure and smaller pore size of the film. It was noted that the effect of alginate supplement on the reduction in the OTR of the BCA membranes was much greater than that in the WVTR. The alginate supplement at 0.75 and 1.0 % (w/v) in culture medium caused a remarkable decline in the OTR to 101 and 57 cc/m^2 day or 0.28 and 0.16% of those of the BC membrane, respectively, however, only minor influence on WVTR was observed. The approximate diameter of water molecule is 0.28 nm,[21] which is only slightly smaller than that of oxygen molecule (0.36 nm).[22] Nonetheless, the decrease in pore size of the BCA film did not have a strong effect on WVTR results. This could

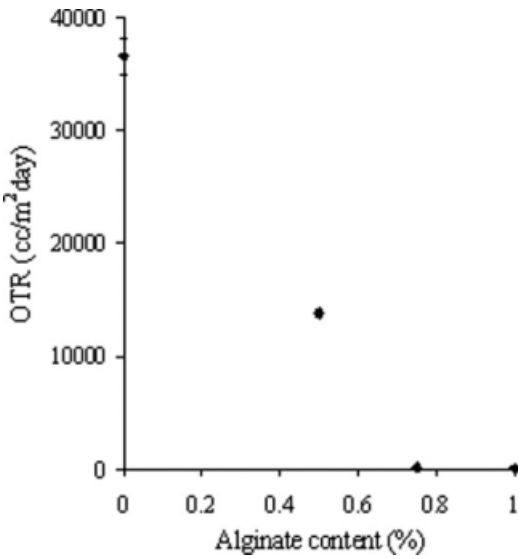

Figure 12 The oxygen transmission rate (OTR) of the BC and BCA membranes as a function of alginate content (% w/v) in culture medium.

possibly be explained from the hydrophilic characteristics of the films. The BC and BCA films could be swollen by water vapor contained in a feed gas. Solubilization of water into the films enlarged pore diameter and caused a looser fibrous structure of the films. Consequently, it exhibited high-water vapor permeability. This phenomenon has been reported in the other hydrophilic membranes.[23] On the other hand, the OTR analysis was preformed at a relative humidity of 0%; therefore, the fibrous structure during the test should be tighter and less porosity. As a result, the presence of alginate-filled pores caused a considerable decrease in oxygen permeability. It was found that for the BC membrane, the WVTR was less than 1/15 of the OTR but for the 1.0% Al-BCA membrane, the WVTR was more than 30 times the OTR. The results revealed that at 0% relative humidity, the oxygen transfer resistance of the BCA film could be significantly increased by adding sodium alginate into the culture medium during biosynthesis.

CONCLUSIONS

The bacterial cellulose membrane was modified by means of 0.5−1.0% (w/v) alginate supplements in culture medium during its biosynthesis by *Acetobacter xylinum* under static conditions. The cellulose–alginate composite was produced in the form of pellicle that floats on the culture medium surface. The bulk membrane was composed of thin films united together. With the supplement of alginate in the culture medium, the alginate was incorporated into the cellulose fibril network and filled the pores. The presence of alginate-filled pores resulted in the significant reduction of the membrane pore size, denser structure, and improved hydrophilicity. The results from the SEM micrograph and BET revealed that the average pore size significantly decreased when increasing alginate content. The 1% Al-BCA membrane showed 30% higher water absorption and slight reduction in WVTR. The significant decrease of the mechanical strengths dependent on the degree of alginate supplement was observed. The remarkable reduction of OTR of the 0.75−1.0% Al-BCA membranes to 57–101 cc/m^2 day or 0.28%–0.16% of the BC film was achieved. These modified characteristics make the BCA membrane a good candidate for the applications in membrane separation. The further investigation of the BCA membrane for use in pervaporation is ongoing.

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