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Biosynthesis and characterization of bacteria cellulose–chitosan film

Muenduen Phisalaphong *, Nirun Jatupaiboon

Department of Chemical Engineering, Chulalongkorn University, Phaya Thai Road, Bangkok 10330, Thailand

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ABSTRACT

Structure and properties of cellulose film produced by *Acetobacto xylinum* were modified by the supplement of low-molecular-weight chitosan into the culture medium. With the addition of 0.75% (w/v) chitosan, the films of BC-chitosan (BCC) of MW 30,000 and BCC of MW 80,000 were homogeneous with a significantly denser fibril structure, smaller pore diameter and higher surface area in comparison to those of BC films. The pore sizes of the dried films of BC, BCC of MW 30,000 and BCC of MW 80,000 were 224, 151 and 132 Å, respectively. The FTIR spectra indicated the intermolecular interaction between BC and chitosan. The mechanical properties and the water absorption capacity of films were significantly improved by 1.4–1.6 and 1.3–1.4-folds, respectively. However, the addition of chitosan of low-molecular-weight in the dilute concentration as in this study showed no significant influence on some properties of the films such as water vapor transmission rates, average crystallinity index and anti-microbial ability.

1. Introduction

Cellulose, the basic material of all plant substances, is the most abundant polysaccharide found in nature. Cellulose derived from plant is unpurified cellulose associated with other kinds of natural fiber like lignin and hemicellulose while bacterial cellulose (BC) is nearly-purified cellulose. BC can be extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobactor xylinum*, using glucose as a common substrate. Plant-derived cellulose and BC have the same chemical structure. However, with an ultra-fine network structure, BC displayed advantages superior to the counterpart from plants with its physical and chemical properties: such as mechanical strength, crystallinity and hydrophilicity. Several applications of BC in medical fields have been reported such as artificial skin for humans with extensive burns (Fontana et al., 1990), artificial blood vessels for microsurgery (Klemm, Schumann, Udhardt, & Marsch, 2001), scaffolds for tissue engineering of cartilage (Svensson et al., 2005) and wound-dressing (Czaja, Krystynowicz, & Bielecki, 2006). BC shows high water content, good sorption of liquids, non-allergenic and can be safely sterilized without any change to its characteristics. Our previous report (Sanchavanakit et al., 2006) found that BC film supported the growth, spreading and migration of human keratinocytes but not those of human fibroblasts. To extend the field of the potential applications, modifications of BC in physical and biological properties need further studies. The modification by combination with other organic polymers could be an effective method to improve the characteristics and structure of the BC film.

Chitin is recognized as the second most abundant biopolymer in nature. Chitosan is an amino-polysaccharide obtained by the deacetylation of chitin. The structure of chitosan composes of β-(1, 4)-linked GlcN and GlcNAc units. Chitosan can dissolve in dilute acids such as acetic acid, formic acid and so on. Owing to its biodegradability, biocompatibility and non-toxicity, it has been considered as one of the most promising materials (Rinaudo, 2006; Yin, Luo, Chen, & Khutoryanskiy, 2006). Chitosan has been documented for its applications in various fields including the biomedical area (Rinaudo, 2006). It has been known for its absorption of exude, anti-fungal, anti-microbial, anti-viral and wound-healing properties (Jeon, Park, & Kim, 2001; Wu et al., 2004; Liu et al., 2006).The main factors affecting the antibacterial activity of chitosan are molecular weight (MW) and concentration (Jeon et al., 2001; Liu et al., 2006). Chitosan is useful as a wound management aid to reduce scar tissue. Chitosan has also been found to be a good support material for gene delivery, cell culture, tissue engineering, drug delivery, anti-microbial agents and adsorption agents (Jayakumar, Prabaharan, Reis, & Mano, 2005).

The blending of polymers to improve their chemical and physical properties has been received extensive attention in the past several decades (Yin et al., 2006; Wu et al., 2004; Fu & Cheng, 2006; Suto & Ui, 1996; Pawlak & Mucha, 2003; Yang, Hsiao, & Chen, 2002; Hong et al., 2006; Li, Zhuang, Liu, Guan, & Yao, 2002; Shih & Huang, 2003; Lee, Kim, & Kim, 1994). Since the chemical structure of chitosan backbone is very similar to that of cellulose, it was expected that chitosan could be miscible with cellulose and the blending might improve the chemical, physical, mechanical and

* Corresponding author. Tel.: +662 218 6875; fax: 662 218 6877.
E-mail address: muenduen.p@chula.ac.th (M. Phisalaphong).

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biological properties of the developed film. The chemical cross-linking of chitosan and hydroxypropyl cellulose (HPC) blends with glyoxal and glutaraldehyde was reported (Suto & Ui, 1996). In addition, it was found that the chitosan–HPC blends formed transparent homogeneous films with the strong hydrogen bonding interactions between the functional groups of the polymers (Pawlak & Mucha, 2003). Yang et al. (2002) reported that the tensile strength of the composite cellulose membranes coated with different chitosan concentrations increased with increasing chitosan concentration. Furthermore, it was reported that chitosan and cellulose blend membranes demonstrated effective anti-microbial capability against *Escherichia coli* and *Staphylococcus aureus* (Wu et al., 2004).

Considering that chitosan is a disintegrating polysaccharide with a similar structure to that of cellulose, the supplement of chitosan during *A. xylinum* cultivation is proposed in this study to develop a new nanostructure film composed of chitosan and cellulose. Microstructure and mechanical properties of the developed films are then characterized to provide indications for the modification of bacterial cellulose–chitosan film. To the best of our knowledge, this is the first time, in this work, that this type of film and its characteristic structure are reported.

2. Materials and methods

2.1. Microbial strains

The *A. xylinum* strain was isolated from nata de coco. The stock culture was kindly supplied by Pramote Tammarate, the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

2.2. Culture media and method

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 chitosan content. The 75 mL of activated medium was inoculated in a Petri dish 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 was air-dried at room temperature (30 °C) and stored in plastic film before use.

2.3. Characterization of membranes

2.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used primarily to identify the chemical structure of the membrane. The FTIR spectra of the membranes were measured at wave numbers ranging from 4000 to 400 cm^{-1} with a Nicolet (United States) SX-170 FTIR spectrometer.

2.3.2. Water absorption capacity (WAC)

To determine the WAC, the dried membranes were immersed in DI water at room temperature 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:

$$\mathrm{WAC}(\%) = \frac{W_\mathrm{h} - W_\mathrm{d}}{W_\mathrm{d}} \times 100$$

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

2.3.3. Tensile property testing

All the membranes under the study in dry and re-swollen forms were tested for tensile strength, Young's modulus and elongation at break. The film samples were cut into strip-shaped specimens 10 mm width and 10 cm long. The maximum tensile strength and break strain of RBC films were determined with a Lloyd (Southampton, UK) 2000 R universal testing machine. The test conditions followed ASTM D 882. The tensile strength and break strain were the average values determined from 10 specimens.

2.3.4. Scanning electron microscopy (SEM)

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.

2.3.5. Water vapor permeability measurement

The water vapor transmission rate (WVTR) of the dry BC and BCC films with area of 50 cm^2, were determined with a Lyssy (Switzerland) L80-4000 water vapor permeation tester. The test conditions followed ISO 15106-1. The determination of WVTR was done under 38 °C and 90% relative humidity. 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.

2.3.6. 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 up to 373 K for 2 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.

2.3.7. 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 (Phisalaphong, Suwanmajo, & Sangtherapitikul, 2008).

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

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

3. Results and discussion

In static conditions, bacterial cellulose (BC) was synthesized in the form of a pellicle on the surface of a culture medium. The result of our preliminary test demonstrated that adding chitosan of MW 30,000 and 80,000 with the degree of deacetylation (DAC) 0.85 more than 0.75% (w/v) in the culture medium strongly inhibited the growth and BC formation of *A. xylinus*. Moreover, the inhibition effect was considerably enhanced when chitosan of MW 200,000

was applied (data not shown). Therefore, the study of the chitosan supplement was limited to the test with chitosans of MW 30,000 and 80,000 in the concentration range of 0–0.75% (w/v). The structure, pore morphology, tensile strength, chemical structure and anti-microbial ability of the developed films were then examined to investigate the effect of chitosan supplement.

3.1. Surface morphology

Bacterial cellulose–chitosan film was developed by means of adding chitosan into the culture medium during the synthesis by *A. xylinum*. The surface structures of films were then analyzed by scanning electron microscopy (SEM). In this study, BCC and BC film refers to BC film with and without the addition of chitosan in culture medium, respectively. BCC-MW 30,000 and 80,000 referred to sample of BCC films with the supplement of chitosan of MW 30,000 and 80,000, respectively. Fig. 1 reveals the well-organized fibril networks of BC and BCC films. By adding 0.75% chitosan, the obtained films were significantly denser as demonstrated in Fig. 2 for BCC-MW 30,000 and Fig. 3 for BCC-MW 80,000. The apparent BCC films were thicker and denser related to the chitosan content (data not shown).

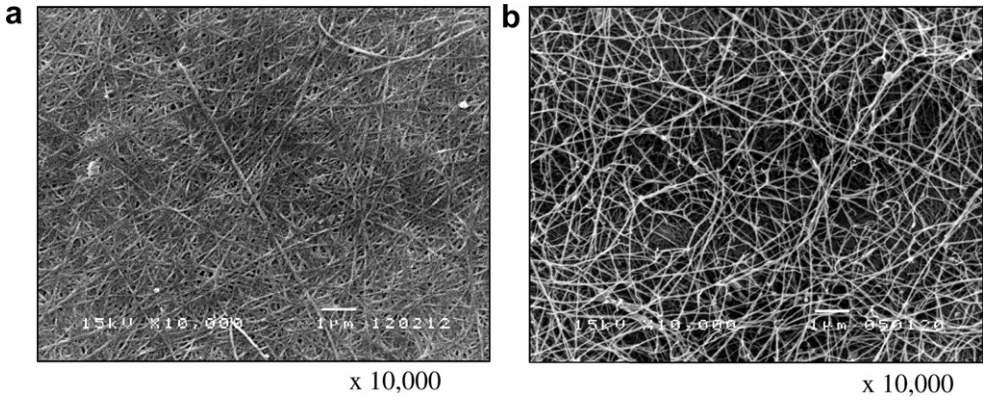

Fig. 1. SEM images of surface morphology of BC film in: (a) dry form and (b) re-swollen form.

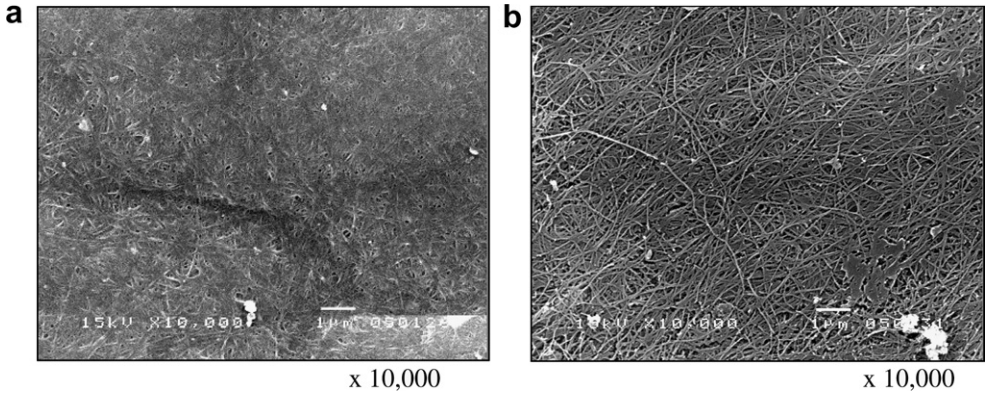

Fig. 2. SEM images of surface morphology of BCC-MW 30,000 film in: (a) dry form and (b) re-swollen form.

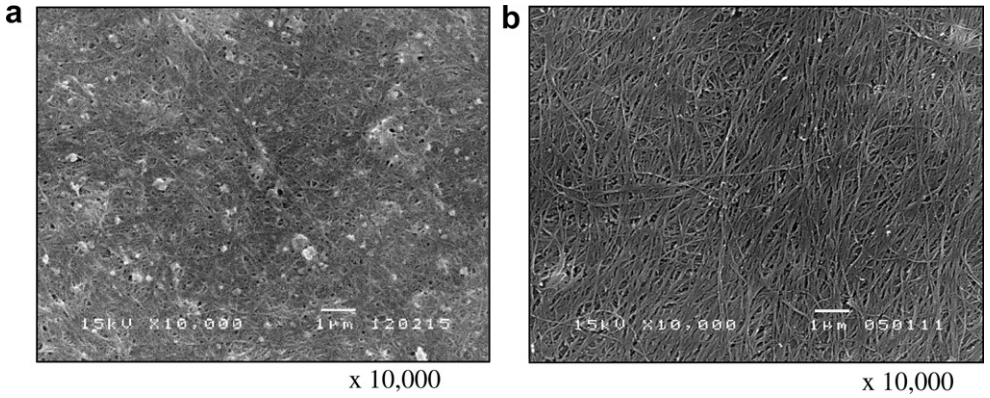

Fig. 3. SEM images of surface morphology of BCC-MW 80,000 film in: (a) dry form and (b) re-swollen form.

3.2. FTIR analysis

FTIR spectroscopy has often been utilized as a useful tool in determining specific functional groups or chemical bonds that exist in a material (Lee et al., 1994). Fig. 4 demonstrated the FTIR spectra of BCC films in the wave numbers ranging from 2800 to 1200 to show bands for amide group, which existed in chitosan molecule, appeared around wave number 1375, 1560 and 1650 cm^{-1} and a band at around 1639 cm^{-1}, which was attributed to glucose carbonyl of cellulose.

The expansion of the FTIR spectra of BCC films at wave number ranging from 1800 to 1500 cm^{-1} in Fig. 5 demonstrated the adsorption bands at around 1639.1 cm^{-1} and around 1557–1560 cm^{-1}. The intense absorption in the spectrum of the cellulose was the band at 1639.1 cm^{-1}, which was mostly assigned to glucose carbonyl of cellulose as shown in Fig. 5a. The characteristic absorption of the chitosan was the band at 1556 cm^{-1}, which was assigned to the amino groups of chitosan as shown in Fig. 5h. The spectrums of the amino groups of chitosan shifted from 1556 cm^{-1} to 1559.5 cm^{-1} and 1559.8 cm^{-1} with the addition of 0.75% chitosan of MW 30,000 and 80,000, respectively. In addition, the intensity of this band increased gradually with chitosan content. The results suggested that intermolecular hydrogen bonding interaction took place between cellulose and chitosan, leading to a good miscible film. A similar observation was described earlier (Yin et al., 2006) in blends of chitosan with two cellulose ethers – hydroxypropylmethylcellulose and methylcellulose by casting from acetic acid solutions. It has been proposed that the intermolecular hydrogen bonding of cellulose was broken down to form cellulose–chitosan hydrogen bonding; on the other hand, the intra-molecular and intra-strand hydrogen bonds held the network flat (Wu et al., 2004). With the introduction of chitosan into the cellulose material, there was a strong interaction between chitosan and cellulose, and a lot of hydrogen bonds, ionic bonds and a few covalent bonds were presented (Fu & Cheng, 2006; Urreaga & de la Orden, 2006).

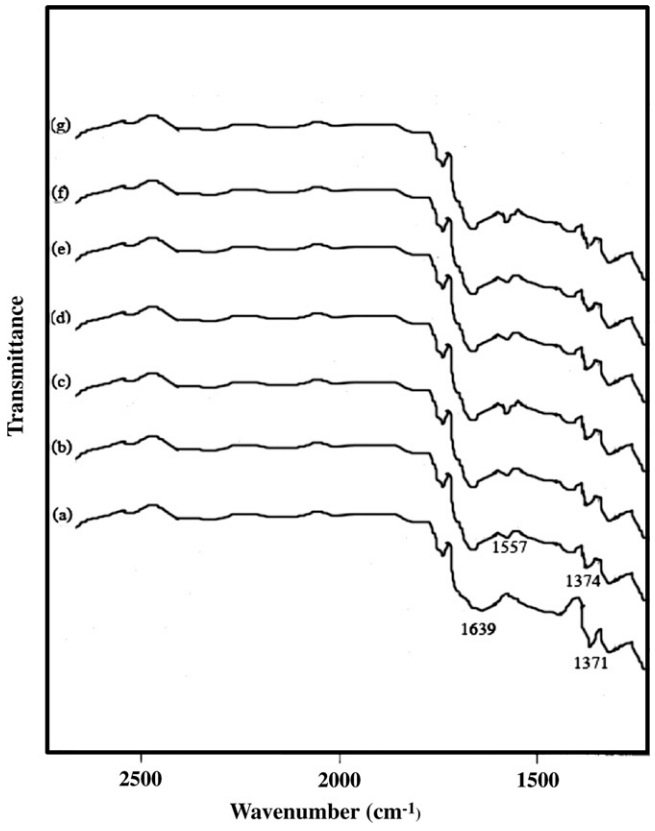

Fig. 4. The FTIR spectra of (a) BC and (b–g) BCC films in wave number ranging from 2800 to 1200 cm^{-1}. The supplements of chitosan (% w/v) in BCC-MW 30,000 were: (b) 0.25%, (c) 0.50% and (d) 0.75%. The supplements of chitosan (% w/v) in BCC-MW 80,000 were: (e) 0.25%, (f) 0.50% and (g) 0.75%.

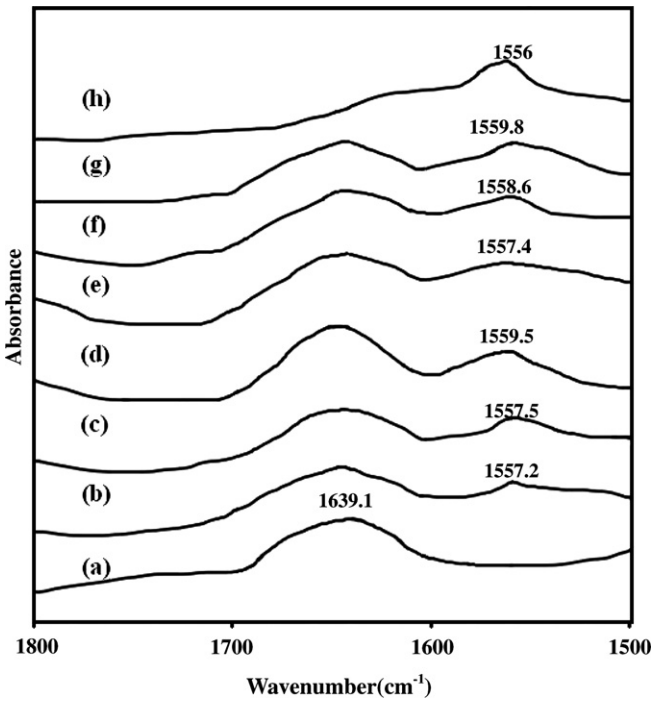

Fig. 5. The FTIR spectra of (a) BC, (b–g) BCC and (h) chitosan films in wave number ranging from 1800 to 1500 cm^{-1}. The supplements of chitosan (% w/v) in BCC-MW 30,000 were: (b) 0.25%, (c) 0.50% and (d) 0.75%. The supplements of chitosan (% w/v) in BCC-MW 80,000 were: (e) 0.25%, (f) 0.50% and (g) 0.75%.

3.3. Mechanical property

Fig. 6 shows the effects of chitosan addition on the tensile strength of the films. The blank sample of the BC film is the sample with 0% of chitosan content. In comparison to that of the BC film, the tensile strength of BCC films increased with an increase of chitosan content. The maximum tensile strength of the dry films

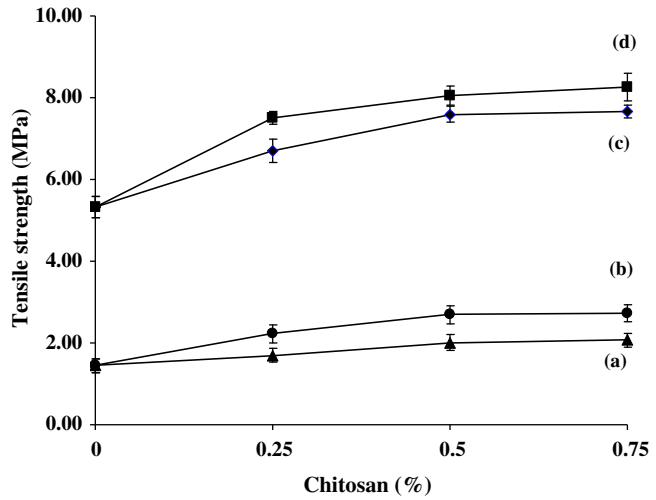

Fig. 6. The tensile strength of BCC films in re-swollen (wet) form: (a) BCC-MW 30,000 and (b) BCC-MW 80,000 and in dry form (c) BCC-MW-30,000 and (d) BCC-MW 80,000 as a function of chitosan content (% w/v) in culture medium.

with an average thickness of 0.037 mm were 7.66 and 8.26 MPa or an increase by 1.4- and 1.6-folds after adding 0.75% (w/v) chitosan of MW 30,000 and 80,000, respectively. The tendency of tensile strength of the re-swollen (wet) films rather corresponded to the dry films but in a lower range. The maximum average values of the wet films were 2.16 and 2.34 MPa after 0.75% (w/v) additions of chitosan of MW 30,000 and 80,000, respectively. The tensile strength of the BCC-MW 80,000 was relatively higher than that of BCC-MW 30,000.

Fig. 7 shows the effects on the Young's modulus. The Young's modulus increased with the increase of chitosan supplement correspondingly to the effect on tensile strength. The maximum average values of Young's modulus of the dry films were 195.0 and 221.8 MPa or an increase by 1.2- and 1.4-folds after 0.75% (w/v) additions of chitosan of MW 30,000 and 80,000, respectively, whereas those values of the wet films were 33 and 36 MPa, respectively.

The investigation of coated composite cellulose–chitosan membranes (Yang et al., 2002) revealed that tensile strength increased with increasing chitosan concentration. Since, the cellulose fiber became thicker after being coated with chitosan, the fiber in the composite membrane could withstand a stronger pull force than the cellulose fiber alone. In this present work, we observed the combination of chitosan with cellulose networks of the film resulting in a denser fiber structure which improved the mechanical strength of BCC films. The tensile strength and Young's modulus in a wet state was much lower than that in a dry state due to the swelling of cellulose fiber and chitosan in an aqueous solution.

Fig. 8 shows the effects of chitosan addition on the percentage of elongation at break. Opposite to the effect on tensile strength, the percentage of elongation at break decreased with increasing chitosan concentration. The minimum percentages of elongation at break of the dry films were 1.91 and 1.44 after 0.75% (w/v) supplements of chitosan of MW 30,000 and 80,000, respectively. We also observed that the elongation at break in a wet state was relatively higher than that of a dry state. The percentages of elongation at break of the wet films were 5.76 and 4.36 after 0.75% (w/v) additions of chitosan of MW 30,000 and 80,000, respectively.

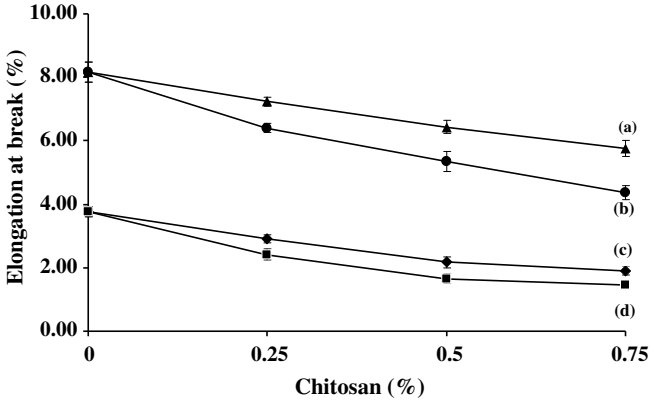

Fig. 8. The elongation at break of BCC films in re-swollen (wet) form: (a) BCC-MW 30,000 and (b) BCC-MW 80,000 and in dry form (c) BCC-MW-30,000 and (d) BCC-MW 80,000 as a function of chitosan content (% w/v) in culture medium.

3.4. Water absorption capacity (WAC)

As can be seen in Fig. 9, the water absorption capacity of BC was 482%; WAC increased with the increase of chitosan content. Supplementation of 0.75% (w/v) of chitosan of MW 30,000 and 80,000 caused the increase in WAC to 606 and 651%, or 1.3- and 1.4-folds in comparison to that of BC, respectively. Chitosan is hydrophilic and can be well incorporated into the cellulose network structure. The increased WAC of the BCC films depended on the amount of chitosan content in the films. The effect of chitosan of MW 80,000 on WAC was relatively higher than that of MW 30,000.

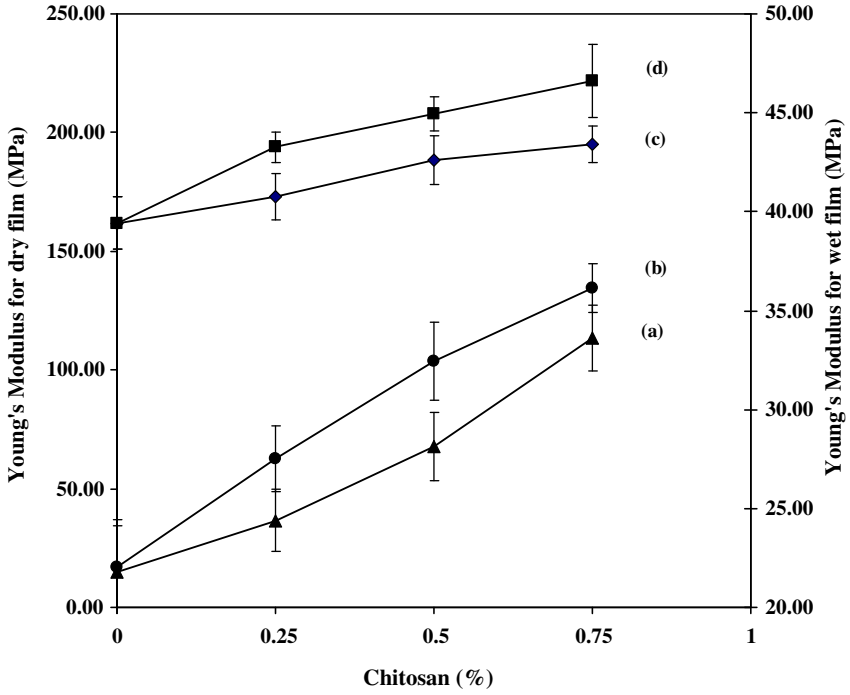

Fig. 7. The Young's modulus of BCC films in re-swollen (wet) form: (a) BCC-MW 30,000 and (b) BCC-MW 80,000 and in dry form (c) BCC-MW-30,000 and (d) BCC-MW 80,000 as a function of chitosan content (% w/v) in culture medium.

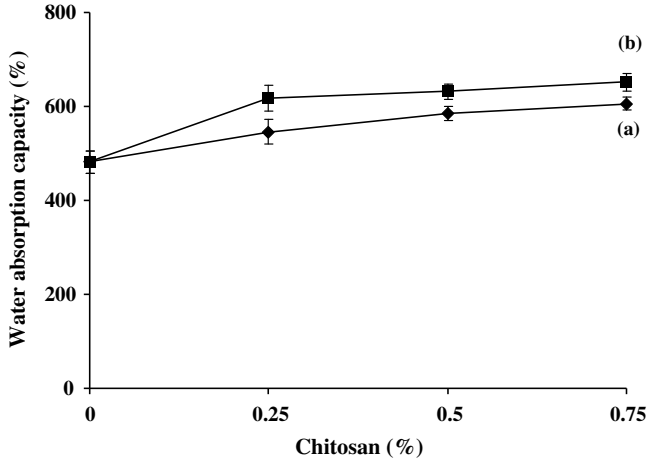

Fig. 9. The water absorption capacity (WAC) of BCC films: (a) BCC-MW 30,000 and (b) BCC-MW 80,000 as a function of chitosan content (% w/v) in culture medium.

3.5. XRD (X-ray diffraction)

The XRD patterns of BC and BCC films are shown in Fig. 10. Generally, the film XRD pattern of BC demonstrated that the peaks observed at 14.4°, 16.6° and 22.4° were attributed to the BC cultured in static circumstance (Hong et al., 2006; Phisalaphong et al., 2008; Keshk & Sameshima, 2006). The broad diffraction peaks observed for BC were because BC is not a completely crystalline material (Hong et al., 2006). The diffractograms of BCC with the supplements of 0.75% (w/v) of chitosan of MW 30,000 and 80,000 showed nearly no difference from that of BC. The average crystallinity index value of BC film was slightly higher than that of BCC film. The average crystallinity index value of the BCC-MW 30,000, BCC-MW 80,000 and BC was 73.04, 74.86 and 75.16, respectively. From the previous report (Fu and Cheng, 2006), the crystallinity of the paper decreased when chitosan was added onto the paper. However, the small amount of low-molecular-weight chitosan added in this study did not considerably influence the formation of crystallites of the films. Similar observation was discussed in the *O*-carboxymethylated chitosan/cellulose blend film (Li et al., 2002).

3.6. Porosity

The total surface area and average pore size of the entire BC film determined by BET were 12.62 m^2/g and 224 Å (Phisalaphong et al., 2008), respectively. As shown in Table 1, BCC films with supplementation of 0.75% (w/v) of chitosan of MW 30,000 and 80,000 had pore sizes smaller than those of BC while the surface areas were relatively increased in the latter. The pore sizes of the BCC-MW 30,000 and 80,000 were 151 and 132 Å, with the total surface area of 14.2 and 14.8 m^2/g, respectively.

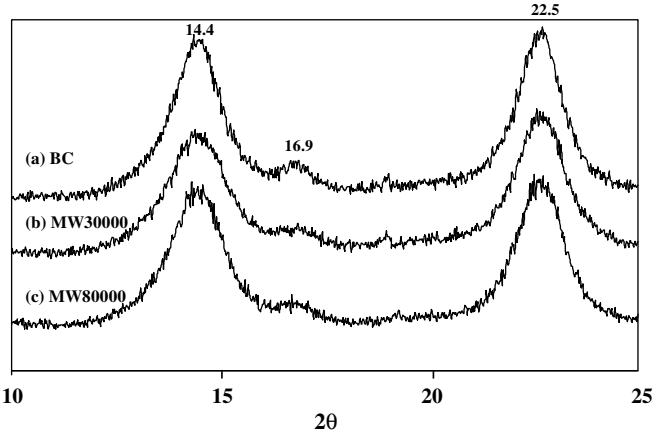

Fig. 10. The X-ray pattern of the films: (a) BC, (b) BCC-MW 30,000 and (c) BCC-MW 80,000.

For the re-swollen form, the total surface area and average pore sizes of the BC film were 55.2 m^2/g, and 612 Å, respectively. The pore sizes of the wet films of BCC-MW 30,000 and MW 80,000 were 486 and 401 Å, with the total surface area of 82.3 and 98.1 m^2/g, respectively. This result was in good agreement with the observation from SEM micrograph. It was found that the additional chitosan of the blended cellulose–chitosan film resulted in the formation of denser matrix networks, higher surface area and smaller pore diameter (Yang et al., 2002).

The distribution range of the pore diameter determined by BET is shown in Table 1. The average pore size determined by BET was much smaller than those on the surface observed from the SEM images. Therefore, the pores inside the film should be smaller than those on the surface.

3.7. Water vapor transmission test

The water vapor transmission rate (WVTR) of BC was 1593 g/m^2 day. The water vapor transmission rates of the BCC-MW 30,000 and MW 80,000 were 1578 and 1564 g/m^2 day, respectively. Therefore, the new network of BCC film had a slightly lower water vapor transmission rate in comparison to the BC film.

3.8. Anti-microbial ability

Escherichia coli and *Staphylococcus aureus* were used for the antibacterial tests of the films. The results revealed that the addition of chitosan of MW 30,000 and 80,000 up to 0.75% (w/v) in the culture medium had no significant impact on the growth of *E. coli* and *S. aureus*. Moreover, from the anti-fungal examination on the growth of *Aspergillus niger*, the anti-fungal activity of BCC film was only slightly improved by adding 0.75% (w/v) of chitosan MW 80,000. Since chitosan blending with other materials could improve their anti-microbial properties (Wu et al., 2004; Li et al., 2002; Shih and Huang, 2003), the supplement of chitosan into cellulose material was expected to introduce anti-microbial abilities into cellulose fiber. Blended film of cellulose with 2–6 wt% *O*-carboxymethylated chitosan of MW 1.08×10^6 (DAC = 0.85) exhibits satisfying antibacterial activity against *E. coli* (Li et al., 2002). However, besides the concentration of chitosan in the material, the anti-microbial ability of films composed with chitosan should rely on their cationic nature, molecular size (Liu et al., 2006) and DAC of the applied chitosan. In this study, the small amount of low-molecular-weight chitosan existing in the BCC films did not exhibit major change in anti-microbial activity of the film.

Table 1
Pore diameter, average pore size and surface area of the BC and BCC analyzed with BET analyzer

Film sample	Pore diameter (Å)	Average pore diameter (Å)	Surface area (m^2/g)
BC (dry form)	45–800	224	12.6
BCC-MW 30,000 (dry form)	20–700	151	14.2
BCC-MW 80,000 (dry form)	20–600	132	14.8
BC(re-swollen form)	55–1000	612	55.2
BCC-MW 30,000 (re-swollen form)	50–1000	486	82.3
BCC-MW 80,000 (re-swollen form)	50–900	401	98.1

4. Conclusions

Modifying bacterial cellulose by means of adding 0.25–0.75 (% w/v) of chitosan (85% DAC) of MW 30,000 and 80,000 in the culture medium during biosynthesis by *A. xylinum* caused a number of valuable features including high mechanical properties in a wet and a dry state, a high water absorption capacity (WAC) and high average surface area. Although the solubility of the chitosan powder in 1% acetic acid solution was approximately 1.0% (w/v), the BCC film did not dissolve in 1% acetic acid solution. Moreover, the FTIR spectra of the BCC films indicated intermolecular interaction between the hydroxyl groups of cellulose fiber and the amino groups of chitosan. The films of BCC-MW 30,000 and 80,000 films were homogeneous with a significantly denser fibril structure, smaller pore diameter and higher surface area in comparison to those of BC films. Therefore, chitosan should be well-bonded with the cellulose molecule chains in form of bio-copolymerization of cellulose and chitosan fibril network. However, the addition of chitosan of low-molecular-weight in the dilute concentration as in this study showed no significant influence on some properties of the films such as water vapor transmission rates, average crystallinity index and anti-microbial ability.

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References

Czaja, W., Krystynowicz, A., & Bielecki, S. (2006). Microbial cellulose the natural power to heal wounds. *Biomaterials, 27*, 145–151.

Fontana, J. D., de Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., et al. (1990). Acetobacter cellulose pellicle as a temporary skin substitute. *Applied Biochemistry and Biotechnology* (24/25), 253–263.

Fu, L.-H., & Cheng, J.-Q. (2006). Effect of interaction between chitosan and cellulose on the paper property. *Zhongguo Zaozhi Xuebao/Transactions of China Pulp and Paper, 21*, 48–51.

Hong, L., Wang, Y. L., Jia, S. R., Huang, Y., Gao, C., & Wan, Y. Z. (2006). Hydroxyapatite/bacterial cellulose composites synthesized via a biomimetic route. *Materials Letters, 60*, 1710–1713.

Jayakumar, R., Prabaharan, M., Reis, R. L., & Mano, J. F. a. (2005). Graft copolymerized chitosan – Present status and applications. *Carbohydrate Polymers, 62*, 142–158.

Jeon, Y.-J., Park, P.-J., & Kim, S.-K. (2001). Antimicrobial effect of chitooligosaccharides produced by bioreactor. *Carbohydrate Polymers, 44*, 71–76.

Keshk, S., & Sameshima, K. (2006). Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme and Microbial Technology, 40*(1), 4–8.

Klemm, D., Schumann, D., Udhardt, U., & Marsch, S. (2001). Bacterial synthesized cellulose-artificial blood vessels for microsurgery. *Progress in Polymer Science, 26*, 1561–1603.

Lee, Y. M., Kim, S. H., & Kim, S. J. (1994). Preparation and characterization of β-chitin and poly (vinyl alcohol) blend. *Polymer, 37*, 5897–5905.

Li, Z., Zhuang, X. P., Liu, X. F., Guan, Y. L., & Yao, K. D. (2002). Study on antibacterial *o*-carboxymethylated chitosan/cellulose blend film from LiCl/*N*,*N*-dimethylacetamide solution. *Polymer, 43*, 1541–1547.

Liu, N., Chen, X.-G., Park, H.-J., Liu, C.-G., Liu, C.-S., Meng, X.-H., et al. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers, 64*, 60–65.

Pawlak, A., & Mucha, M. (2003). Thermogravimetric and FTIR studies of chitosan blends. *Thermochimica Acta, 396*, 153–166.

Phisalaphong, M., Suwanmajo, T., & Sangtherapitikul, P. (2008). Novel nanoporous membranes from regenerated bacterial cellulose. *Journal of Applied Polymer Science, 107*, 292–299.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*, 603–632.

Sanchavanakit, N., Sangrungraungroj, W., Kaomongkolgit, R., Banaprasert, T., Pavasant, P., & Phisalaphong, M. (2006). Growth of human keratinocytes and fibroblasts on bacterial cellulose film. *Biotechnology Progress, 22*, 1194–1199.

Shih, C.-Y., & Huang, K. (2003). Synthesis of a polyurethane-chitosan blended polymer and a compound process for shrink-proof and antimicrobial woolen fabrics. *Journal of Applied Polymer Science, 88*, 2356–2363.

Suto, S., & Ui, N. (1996). Chemical crosslinking of hydroxypropyl cellulose and chitosan blends. *Journal of Applied Polymer Science, 61*, 2273–2278.

Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D. L., Brittberg, M., et al. (2005). Cellulose as a potential scaffold for tissue engineering of cartilage. *Biomaterials, 26*, 419–431.

Urreaga, J. M., & de la Orden, M. U. (2006). Chemical interactions and yellowing in chitosan-treated cellulose. *European Polymer Journal, 42*(10), 2606–2616.

Wu, Y.-B., Yu, S.-H., Mi, F.-L., Wu, C.-W., Shyu, S.-S., Peng, C.-K., et al. (2004). Preparation and characterization on mechanical and antibacterial properties of chitsoan/cellulose blends. *Carbohydrate Polymers, 57*, 435–440.

Yang, L., Hsiao, W. W., & Chen, P. (2002). Chitosan–cellulose composite membrane for affinity purification of biopolymers and immunoadsorption. *Journal of Membrane Science, 197*, 185–197.

Yin, J., Luo, K., Chen, X., & Khutoryanskiy, V. V. (2006). Miscibility studies of the blends of chitosan with some cellulose ethers. *Carbohydrate Polymers, 63*, 238–244.