Carbohydrate Polymers 79 (2010) 455–460

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Novo aloe vera–bacterial cellulose composite film from biosynthesis

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ARTICLE INFO

Article history:
Received 23 May 2009
Received in revised form 21 August 2009
Accepted 27 August 2009
Available online 3 September 2009

Keywords:
Bacteria cellulose
Aloe vera
Acetobacter xylinum

ABSTRACT

A novo bio-polymer composite film of cellulose and aloe vera gel was developed by means of adding aloe vera gel in the culture medium during biosynthesis using *Acetobacter xylinum* in static cultivation. The interaction between bacterial cellulose (BC) fibrils and aloe vera gel was illustrated via FTIR analysis. With the 30% v/v aloe gel supplement in the culture medium, a fibre-reinforced bio-polymer film displayed significantly improved properties in mechanical strength, crystallinity, water absorption capacity and water vapor permeability in comparison to those of the unmodified BC film. The average pore size of the modified film either in the dry or re-swollen form was approximately reduced to 1/5 of those of the unmodified BC films with a narrow pore size distribution.

1. Introduction

Cellulose, a linear polysaccharide, is the most abundant organic material with a variety of useful applications. It is found as a structural component, often bound to other polymers (pectin, lignin, hemicellulose, etc.) in the cell wall of plants, algae and also some lower animals. Only a few bacterial species, taxonomically closely related to the genus *Acetobacter xylinum*, produce and extracellularly secrete cellulose in form of fibre. Bacterial cellulose (BC) produced by *A. xylinum*, has unique properties including high water holding capacity, high crystallinity, hydrophilicity, high tensile strength and a highly pure and ultra fine fibre network structure (Iguchi, Yamanaka, & Budhiono, 2000; Wan et al., 2007). Recently, BC has been studied for its use as artificial skin and blood vessels (Klemm, Schumann, Udhardt, & Marsch, 2001) scaffold for tissue engineering of cartilage (Svensson et al., 2005), and wound-dressing (Czaja, Krystynowicz, & Bielecki, 2006). Still, an innovative wound-dressing has been continuously developed in a wide range of good candidate materials such as alginate, polyurethane, chitosan, and aloe vera.

Aloe vera is one of the oldest healing plants known to mankind. Aloe vera gel is the mucilaginous gel obtained from the squeezing of the clear jelly-like substance of the parenchyma tissue. Aloe vera gel has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetize tissue, preclude bacterial, fungal, and viral growth, act as an anti-inflammatory agent and enhance blood flow (Davis, DiDonato, Hartman, & Haas, 1994; Grindlay & Reynolds, 1986; Heggers et al., 1996; Reynolds & Dweck, 1999; Yao et al., 2009). Aloe vera gel was applied to inhibit fibroplasia in wound healing, to promote both tissue growth and differentiation in tissue culture and for the treatment of burn wounds (Reynolds & Dweck, 1999). Acemannan, an ordered linear polymer of substantially acetylated mannose monomers isolated from the aloe vera gel is considered by many to be one of the major active ingredients (Femenia, García-Pascual, Simal, & Rosselló, 2003; Femenia, Sánchez, Simal, & Rosselló, 1999; Ni, Turner, Yates, & Tizard, 2004; Reynolds & Dweck, 1999; Yu, Jin, Xin, & Min, 2009). The activities of acemannan as an antiviral agent, an immunomodulator, an agent in reducing opportunistic infections and stimulating the healing processes were reported (Reynolds & Dweck, 1999; Tai-Nin Chow, Williamson, Yates, & Goux, 2005).

Based on the advantageous properties of BC and aloe vera gel, in this study, a novo nanostructure film composed of BC and aloe vera was developed by the supplementation of aloe vera gel during biosynthesis of the bacteria cellulose film. The surface morphology, pore structure, tensile strength, water absorption capacity (WAC), crystallinity, and water vapor permeability of the modified films were then examined and compared with those of the typical BC film. To the best of our knowledge, this type of blend combination was first prepared in this work.

2. Materials and methods

2.1. Microbial strains

The *A. xylinum* AGR 60 kindly supplied by the laboratory of Pramote Tammarate (The Institute of Food Research and Product Development, Kasetsart University, Bangkok) was used in this study.

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doi:10.1016/j.carbpol.2009.08.039

2.2. Culture media and method

The culture medium was coconut-water supplemented with 5.0% sucrose, 0.5% ammonium sulfate and 1.0% acetic acid. Preculture was prepared by a transfer of 50 mL stock culture to 1000 mL medium in a 1500 mL bottle and incubated statically at 30 °C for 7 days. After that the surface pellicle was removed and the preculture broth of 5% (v/v) was added to the main culture medium with the various aloe vera contents from 0% to 50% v/v. The 75 mL of the activated main culture medium was inoculated in a Preti-dish and kept at 30 °C for 7–10 days. After that, all sample films were purified by washing with deionized (DI) water and then were treated with 1% (w/v) sodium hydroxide solution at room temperature (30 °C) for 24 h to remove bacterial cells followed by a rinse with DI water until pH became neutral. Afterward, the films were air-dried at room temperature and stored in plastic film.

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 2800 to 1200 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:

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

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

2.3.3. Mechanical property

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 the film samples were determined with a Lloyd (Southampton, UK) 2000R 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 film with area of 50 cm^2 and 0.03 mm of thickness was 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. The principle of this electronic tester was similar to that of conventional method. One side of the film was exposed to water vapor. As water solubilized into the film and permeated through the sample material, on the other side of the 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 348 K for 3 h and cooled down to room temperature before the BET analysis. The BET pore size and surface area were determined with N2 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 CuKα, radiation (λ = 1.54 Å). The operating voltage and current were 40 kV and 30 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 & Jatupaiboon, 2008).

3. Results and discussion

A bio-polymer composite of cellulose and aloe vera gel was produced in the form of pellicles that floated on the medium surface during the cultivation of *A. xylinum* using a culture medium containing aloe vera gel. A decline in the pellicle formation rate relating to the ratio of aloe vera gel in the medium culture was observed (data not shown). Since the supplement of aloe vera gel increased the viscosity of the culture medium, it resulted in a decrease in the oxygen transfer rate. *A. xylinum* is an aerobic bacterium; therefore, the reduction of oxygen in the medium could become the limiting factor for cell growth and cell activities.

After the air-drying processes, the film in dry state was obtained namely, bacterial cellulose–aloe vera (BCA) film. It was found that the BCA film formation using the culture medium with 30% (v/v) aloe vera gel addition gave a uniform structure with a slightly lower BC pellicle synthesis rate. However, the addition of more than 30% aloe vera gel caused a considerable decrease of the pellicle formation. The effects of aloe vera gel supplement on the film characteristics such as surface morphology, chemical structure, mechanical strength, water absorption capacity, crystallinity, porous structure and water vapor transmission were then investigated.

3.1. Surface morphology

SEM images in Figs. 1–3 illustrate the differences in the surface morphology of the developed films supplemented with 0%, 30% and 50% (v/v) of aloe vera gel in the culture mediums, respectively. Compared to the dried films, the re-swollen films exhibited a looser fibril network structure according to the high water absorption of the films. In Fig. 2, the film with the supplement of 30% aloe vera gel displayed good incorporation of aloe vera gel into the BC fibril network. However, in the case of the 50% aloe vera gel supplement (Fig. 3), the structure of the film became less uniform with noticeable excessive gel on the film surfaces.

3.2. FTIR analysis

Fourier transform infrared (FTIR) spectroscopy of BC and BCA films was carried out in order to detect the occurrence of new peaks or any peak shift that could be attributed to interactions between cellulose and aloe vera gel. The FTIR spectra of all samples were detected at wavenumbers ranging from 2800 to 1200 cm^{-1}

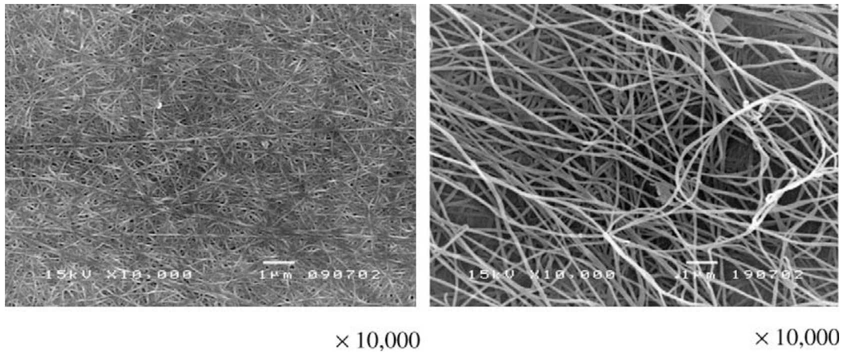

Fig. 1. SEM images of surface morphology of BC film in dry form (left) and re-swollen form (right).

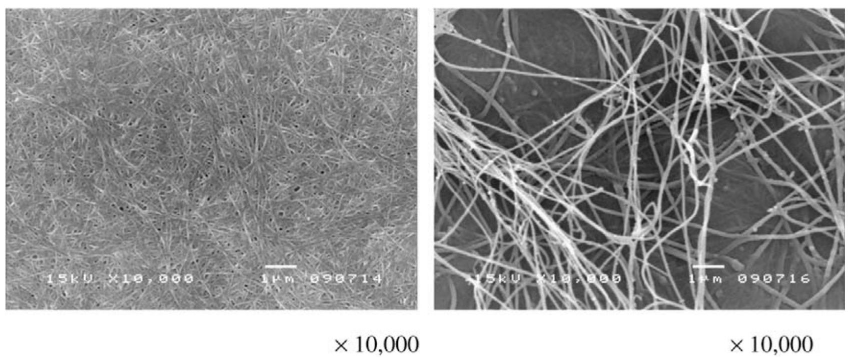

Fig. 2. SEM images of surface morphology of BCA-30% aloe vera film in dry form (left) and re-swollen form (right).

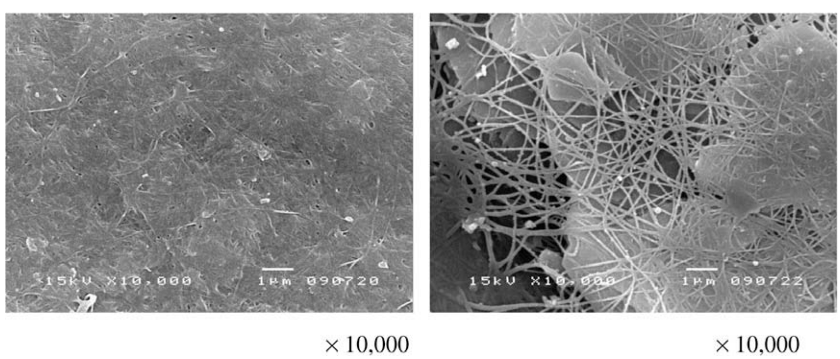

Fig. 3. SEM images of surface morphology of BCA-50% aloe vera film in dry form (left) and re-swollen form (right).

as shown in Fig. 4. For close observation, the expansion of the FTIR spectra of the films at wave number ranging from 1800 to 1500 cm^{-1} was displayed in Fig. 5. In this region, the intense absorption in the spectrum of the cellulose was the band at 1642.9 cm^{-1}, which has been assigned to carbonyl groups (Klemm et al., 2001), while the characteristic absorption of the aloe vera was the band at 1594.1 cm^{-1}, which was assigned to amino groups as shown in Fig. 4(a) and (h), respectively. The bands at 1650–1578 cm^{-1} were assigned to C=O stretching, which overlaps with NH bending. The absorption band at 1565–1540 cm^{-1} was NH deformation. In Fig. 5(b–h), the films developed by the supplement of aloe vera gel of 5%, 10%, 20%, 30%, 40%, and 50% (v/v) in the culture medium present a peak shift and an occurrence of two new peaks from 1562.6 cm^{-1} to 1562.0, 1562.7, 1563.2, 1573.9/1541.7, 1574.3/1541.9, and 1574.6/1541.8 cm^{-1}, respectively. The presence of the two new peaks in FTIR spectra in the region of 1574 (higher frequency) and 1542 cm^{-1} (lower frequency) when the films were developed by the supplement of 30–50% (v/v) aloe vera gel implied the incident of the intermolecular interaction of BC and the amino groups of aloe vera gel. As referred to the SEM analysis, it supported that aloe vera gel could attach and intermolecular bond to the cellulose fibril.

3.3. Mechanical property

Fig. 6 shows the tensile strength of BC and BCA films as a function of aloe vera gel content observed at the average film thickness of 0.030 mm. By supplementation of the gel from 0% to 30% (v/v), it was found that the gel merged well into the fibril network resulting in improvement of the tensile strength of the films from 5.32 to 8.67 MPa in proportion to the gel content. However, with the excessive gel supplements in the range of 40–50% v/v, the pellicle formation rate was significantly decreased and the miscellaneous structure of the films was observed as previously shown in the SEM images. With the gel supplement of 50% (v/v), the tensile strength of the film was drastically reduced to 3.42 MPa.

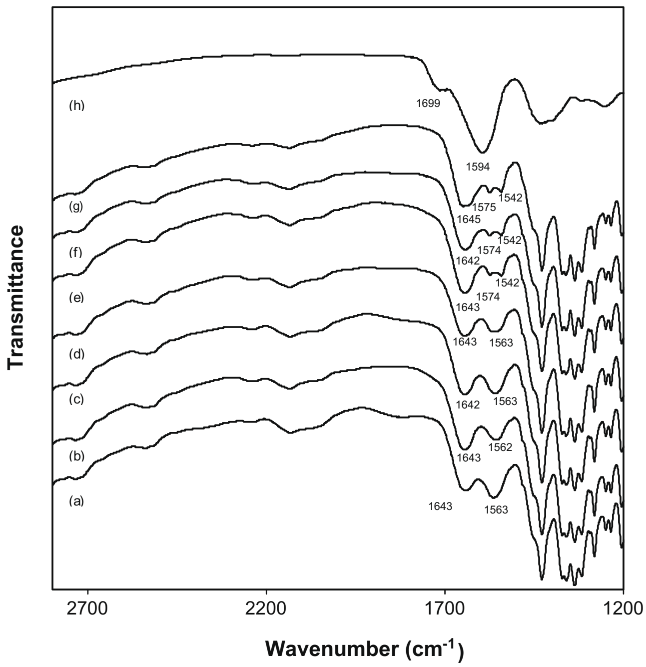

Fig. 4. The FTIR spectra of (a) BC and (b–g) BCA films and (h) aloe vera gel, in wave numbers ranging from 2800 to 1200 cm^{-1}. The supplement of aloe vera gel (% v/v): (b) 5; (c) 10; (d) 20; (e) 30; (f) 40 and (g) 50.

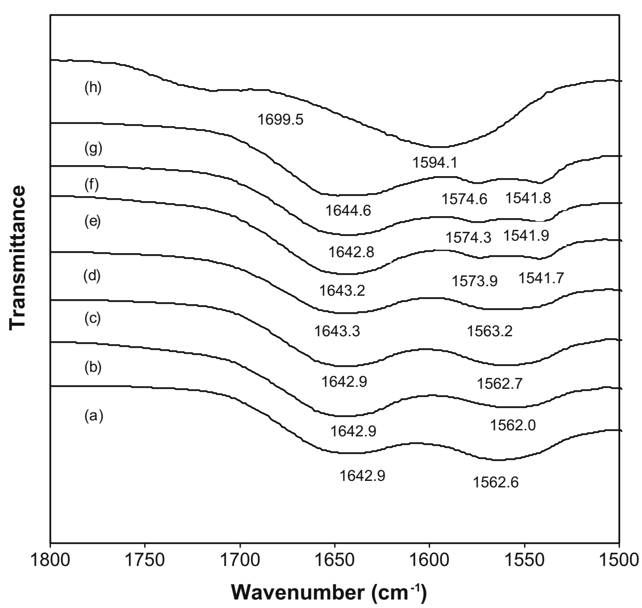

Fig. 5. The FTIR spectra of (a) BC and (b–g) BCA films and (h) aloe vera gel in wave numbers ranging from 1800 to 1500 cm^{-1}. The supplement of aloe vera gel (% v/v): (b) 5; (c) 10; (d) 20; (e) 30; (f) 40 and (g) 50.

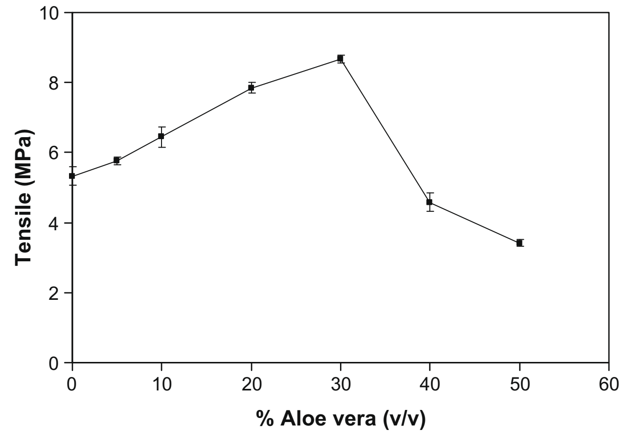

Fig. 6. Tensile strength of the BCA films as a function of aloe vera content (% v/v) in culture medium.

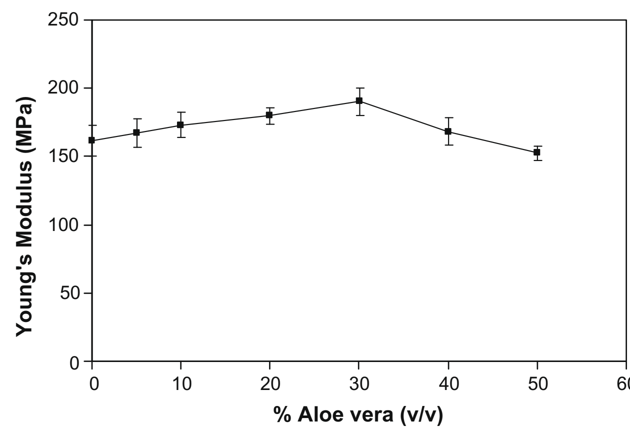

Fig. 7. Young's modulus of the BCA films as a function of aloe vera content (% v/v) in culture medium.

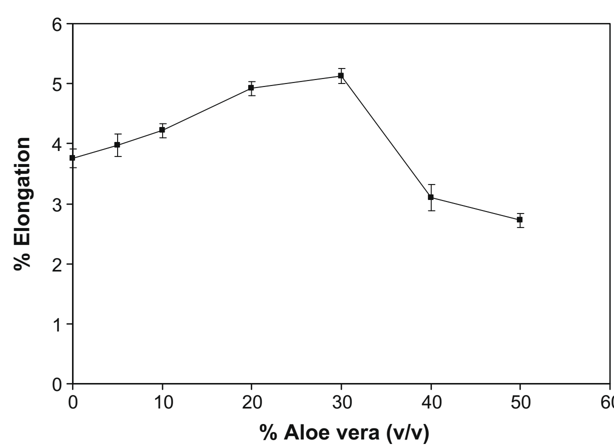

Fig. 8. The elongation at break of the BCA films as a function of aloe vera content (% v/v) in culture medium.

The effect of the aloe vera gel supplement on Young's modulus (Fig. 7) and elongation at break (Fig. 8) were similar to that on the tensile strength. Supplementation of aloe vera gel from 0% to 30% (v/v) increased the Young's modulus of the film from 161.80 to 190.20 MPa. However, the Young's modulus of the film was reduced with the gel supplement of 40–50% (v/v). The elongation at break also increased with the gel supplement from 0% to 30% (v/v) and declined with the surplus supplement to 40–50% (v/v).

The supplement of aloe vera gel at 30% (v/v) in the culture medium yielded about 1.6 times of tensile strength, 1.2 times of Young's modulus and 1.4 times of elongation at break higher than those of the film synthesized in the normal culture medium. By addition of 30% (v/v) aloe vera gel in the culture medium, the developed BCA film was reinforced to withstand stronger pull force than the typical BC film as a result of the formation of the aloe vera gel–cellulose composite film. The combination of crystalline cellulose and amorphous gel at a suitable ratio could form a material with the advantageous properties of strength and stiffness. In addition, the improved mechanical strength of the BCA film could be as a result of the denser structure with smaller pore diameter and narrow pore size distribution of the film.

3.4. Water absorption capacity (WAC)

The effect of the aloe vera gel supplement on the water absorption capacity (WAC) of the films was analogous to that of the mechanical strength (Fig. 9). The aloe vera gel supplementation at 30% v/v improved the WAC of the film to 735% or about 1.5 times of that of the unmodified BC film. Degree of water swelling and WAC of the blend films could be increased due to the introduction of hydrophilic component. On the previous study (Phisalaphong, Suwanmajo, & Tammarate, 2008), the blending of bacteria cellulose with alginate gel resulted in the increase in the water absorption ability of the film. Aloe vera gel is very hydrophilic and could conjugate well into the cellulose network structure; therefore, the WAC of the BCA film was enhanced. On the other hand, the WAC of the film dropped when the aloe vera gel supplement was greater than 30% (v/v). At the excessive gel addition, the developed film structure became miscellaneous and weak; consequently, the water holding capacity of the film decreased.

3.5. XRD (X-ray diffraction)

The X-ray diffraction (XRD) patterns of BC and BCA films are shown in Fig. 10. The XRD pattern of the BC film developed from the normal culture medium demonstrated the peaks observed at 14.56°, 16.87° and 22.74° in the comparable intensities as previously reported for the typical BC cultured in static circumstances (Hong et al., 2006; Phisalaphong & Jatupaiboon, 2008; Phisalaphong et al., 2008). Because BC is not a completely crystalline material, the diffraction patterns from the films show broad peaks. The percentages of aloe vera gel supplement in the range of 0–50% v/v in the culture medium showed no differences in the reflective-angle and *d*-spacing from those of the typical BC film (Table 1). However, with the aloe gel supplement from 0% to 30% (v/v), the crystalline indices (CI) of the films gradually increased from 78.45 to 82.77, respectively. The higher peak intensity could be as a result of the relatively denser structure of the BCA films. In general, the controlling parameters such as the composition of culture media, pH, temperature and oxygen tension could have an effect on bacterial growth and the cellulose biosynthesis. The higher CI of the film could be because of a relatively higher degree of order form of the polymer chains owning to the different culture medium composition. Similar to the mechanical properties, the CI dropped considerably to 69.75 and 68.52 with the aloe vera gel supplement at 40% and 50% (v/v), respectively. The result implied that with the addition of aloe vera gel in the range of 0–30% (v/v), the developed film of BCA was more orderly arranged in uniform and firmly fibre network than those in the range of 40–50% (v/v).

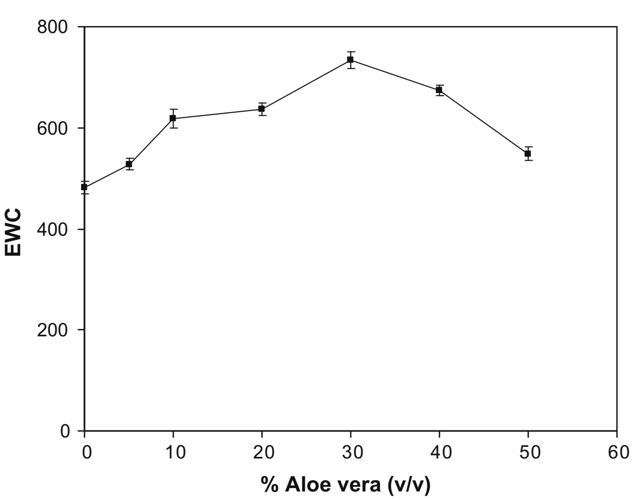

Fig. 9. The equilibrium water content (EWC) of the BCA films as a function of aloe vera content (% v/v) in culture medium.

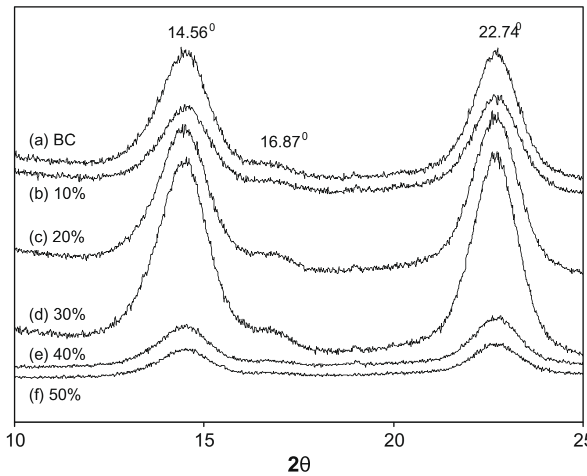

Fig. 10. X-ray patterns of the BC and BCA films: (a) BC; (b) BCA-10% aloe vera; (c) BCA-20% aloe vera; (d) BCA-30% aloe vera; (e) BCA-40% aloe vera; and (f) BCA-50% aloe vera.

Table 1
CI, reflective-angle, *d*-spacing values of the BC and BCA films.

Film sample	CI	2θ [$d(1\,\bar{1}\,0)$]	2θ [$d(1\,1\,0)$]	2θ [$d(0\,2\,0)$]
BC	78.45	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
BCA-10% aloe vera	79.16	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
BCA-20% aloe vera	80.25	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
BCA-30% aloe vera	82.77	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
BCA-40% aloe vera	69.75	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)
BCA-50% aloe vera	68.52	14.56° (6.08)	16.87° (5.25)	22.74° (3.91)

3.6. Porosity

The total surface area and average pore size determined by BET of the BC film were 12.6 m^2/g and 224 Å in dry form and 55.2 m^2/g and 612 Å in re-swollen form, respectively. As shown in Table 2, Figs. 11 and 12, the BCA films had average pore sizes smaller than that of the BC, while the surface area slightly increased. The pore sizes slightly decreased with the increase of aloe vera gel content. Overall, the average pore sizes of the modified films either in dry or re-swollen form were approximately reduced to approximately 1/5

Table 2
Pore diameter and surface area of the BC and BCA films analyzed by BET analyzer.

Film sample	Average pore diameter (Å)	Surface area (m^2/g)
BC (dry form)	224	12.6
BCA-10% aloe vera (dry form)	53	14.2
BCA-30% aloe vera (dry form)	41	15.7
BCA-50% aloe vera (dry form)	38	19.5
BC (re-swollen form)	612	55.2
BCA-10% aloe vera (re-swollen form)	154	59.1
BCA-30% aloe vera (re-swollen form)	150	62.4
BCA-50% aloe vera (re-swollen form)	138	65.2

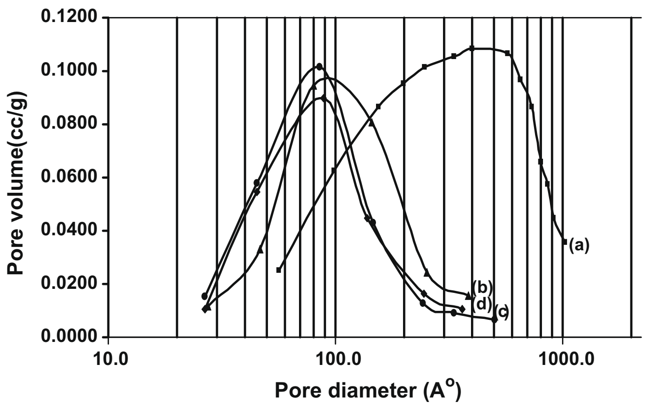

Fig. 11. The pore size distribution of the BC and BCA films in re-swollen form: (a) BC; (b) BCA-10% aloe vera; (c) BCA-30% aloe vera; (d) BCA-50% aloe vera.

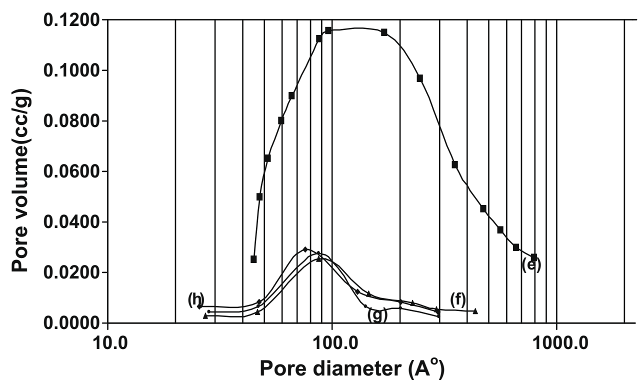

Fig. 12. The pore size distribution of the BC and BCA films in dry form: (e) BC; (f) BC-10% aloe vera; (g) BC-30% aloe vera; (h) BC-50% aloe vera.

of those of the unmodified BC films. The results indicated the occurrence of gel coating and filling pores of the films. The BET results were in accord with the observation from the SEM micrographs.

3.7. Water vapor transmission test

Corresponding to the result regarding water absorption capacity (WAC), the WVTR increased with the addition of aloe vera gel in the range of 0–30% (v/v), and then it decreased with the addition of aloe vera gel at 50% (v/v). The water vapor transmission rate (WVTR) of the BCA film at the aloe vera gel supplement of 0%, 10%, 30%, and 50% (v/v) were at 1616.5, 1821, 2029.5, and 1066 g/m^2 day, respectively. The improved water vapor permeability could be according to the increase of swelling degree and equilibrium water content of the films. A similar result was previously observed in the blended cellulose–alginate membrane (Phisalaphong et al., 2008).

4. Conclusions

The modification of BC film by means of adding aloe vera gel in the culture medium during biosynthesis using *A. xylinum* provided many advantageous properties. The FTIR spectra of the modified films revealed the intermolecular interaction of BC and the amino groups of aloe vera gel. The significant improvement in terms of mechanical properties, water absorption capacity (WAC), water vapor transmission rate (WVTR) and crystalline index were apparently obtained by the addition of up to 30% (v/v) of aloe vera gel in the culture medium. However, the introduction of more than 30% (v/v) aloe vera gel in the culture medium inhibited the film formation and the developed films exhibited miscellaneous structures with inferior film properties. On the nature of biocompatibility of BC and aloe vera gel and on the excellent physical properties of the BCA film, a wide range of applications of the BCA film in medical areas is expected. Investigation of the BCA as materials for tissue engineering is on going.

Acknowledgments

We thank the Thailand Research Fund (TRF) and Chulalongkorn University (RSA5080011) for financial support, the Thailand-Japan Technology Transfer Project (TJTTP-OECF) for the support of analytical equipments.

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