Effect of chitosan penetration on physico-chemical and mechanical properties of bacterial cellulose

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Abstract−Bacterial cellulose-chitosan composites (BC-Ch) were prepared in order to obtain the BC-Ch composites with improved physico-mechanical characteristics. BC sheets were immersed in a Ch solution hoping that the Ch penetrates into the BC sheet. Ch penetration was observed according to variations in temperature, operation mode and treatment time. The morphological changes due to enhanced penetration were observed through FE-SEM, FT-IR and XRD analysis. FE-SEM analyses confirmed the formation of three dimensional multilayered structures in BC-Ch, whose thickness increased with Ch penetration. The FT-IR analysis showed intermolecular hydrogen bonding interaction between the BC and Ch molecules. XRD results revealed a slight decrease in the crystallinity index of the BC-Ch composites compared to pure BC. The mechanical properties, water holding capacity (WHC) and water release rate (WRR) of the BC-Ch composites were significantly improved compared to pure BC. The superior mechanical properties, WHC and water release rate would make the BC-Ch composites suitable for wound dressing and other biomedical applications.

Key words: Bacterial Cellulose, Chitosan, Composites, Physico-mechanical Properties

INTRODUCTION

Plants are the common source of cellulose; however some bacterial species are also capable of producing cellulose called as bacterial cellulose (BC) [1-3]. BC is a pure biopolymer, and exhibits a higher degree of polymerization and better crystallinity than the plant cellulose [4]. The micro fibril of the BC forms reticulate structures which is stabilized by intra and inter molecular hydrogen bonds [5]. The distinctive characteristics of the BC such as its high mechanical strength, water holding capacity, etc. has caused it to be used in a number of commercial products such as tires, headphone membranes, paper, textiles etc. [6,7]. BC also has been applied in the medical field. One of the most pronounced applications is its use as a temporary skin substitute for patients with burns and ulcers [8,9]. BC shows a potential to be used as a proficient wound dressing material because of an easy and safe sterilizing process [10]. The wound healing process is greatly affected by infections caused by some microorganisms. Thus, a wound dressing material should possess an antimicrobial activity and show resistance to the growth of microbial cells [10]. Pure BC itself has no antimicrobial activity to prevent wound infection [11]. BC shows no antioxidant activity which is also required in a secure wound healing process.

Chitosan (Ch) is another abundant polysaccharide present in nature. The chemical structure of chitosan is nearly similar to that of cellulose, except for the replacement of one NH_2 group instead of OH on the glucose molecule. It is highly soluble in dilute acids and biocompatible and also increases membrane permeability [12,13]. Chitosan has some advantages due to its non-toxicity and biodegradability without damaging the environment. Chitosan is a biocompatible material that breaks down slowly to harmless products

of oligomers. These are absorbed entirely in a human body. The degraded chitosan products accelerate the wound healing and are also found to be effective in regenerating the skin tissues of the wound area [14]. Chitosan has attracted a tremendous interest due to its biological activities such as the antimicrobial, immunity enhancing and antitumor effects, drug delivery, and metal adsorption [15]. Studies on its biological activities have proven that it has antimicrobial activities against bacteria, yeast, and fungi. Chitosan is useful as a wound management aid to reduce a scar tissue. The main factors affecting the antibacterial activity of chitosan are its molecular weight (MW) and concentration [16].

Physico-mechanical, chemical, and biological characteristics of a polymer can be enhanced by coating with another polymer or by blending their solutions to produce a composite [11]. The inspiring mechanical properties and reinforcing potentials, abundance and biodegradability of BC nanocrystals make them perfect candidate for the processing of polymer nanocomposites [17]. The ultra structural analysis of BC shows that the chains of the polymers are bound through hydrogen bonding [18]. Therefore, other substances can interact with the BC chains through hydrogen bonding. The deficiencies such as no antioxidant capability, wound healing property, and antimicrobial activity can be overcome by forming its composite with other polymeric substances. Chitosan, having all these characteristics and many hydrogen bonding sites, is an ideal material to be used in the formation of the BC composite.

Efforts have been made to synthesize BC-Ch composites in order to enhance the physical, chemical, and biological activities of BC. In one approach, a BC-Ch composite had been synthesized with the initial addition of Ch to the culture medium. The resulting composite showed better characteristics than the pure BC. However, being antibacterial in nature, Ch strongly inhibited the activity of the bacterial cell resulting in lower production. Moreover, the amount of Ch in the BC-Ch composite was also small in the composite [10,

14]. Another approach applied for BC-Ch preparation was the immersion of a prepared BC into Ch solution at room temperature for few hours [11]. This approach was relatively better because the composites had a higher percentage of Ch and resulted in improved characteristics. The penetration of Ch in the BC-Ch composite would have a definite impact on the physical and biological characteristics of the composites. Therefore, it is necessary to investigate the progress of Ch penetration inside the BC sheets and to determine the effect of Ch penetration on the physicochemical characteristics of the BC-Ch composite. Some physical properties of this type of BC-Ch composite like water holding capacity (WHC) and water release rate (WRR), which has a great impact in biomedical applications, should be investigated.

In the present work, BC-Ch composites were prepared by treating the produced BC sheets with Ch solution. The Ch penetration was evaluated based on different factors such as variable temperature, increased treatment time, operating modes (static and shaking), etc. The effects of Ch penetration on the physic-chemical and mechanical characteristics like WHC, water retention property, tensile strength etc were determined.

MATERIALS AND METHODS

1. Microorganism and Cell Culture

G. hansenii PJK (KCTC 10505BP) was grown on a basal medium containing glucose 10 g/L, yeast extract 10 g/L, peptone 7 g/L, acetic acid 1.5 mL/L, and succinate 0.2 g/L dissolved in 1 L of distilled water [19]. The agar plates used for keeping strains were prepared by dissolving agar 20 g/L in the basal medium. The pH of the medium was adjusted to 5.0 with 1 N NaOH. The prepared basal medium was autoclaved for 15 min at 121 °C. Colonies of *G. hansenii* PJK were inoculated into a 50 mL medium in a 250 mL flask shaken at 150 rpm and cultured at 30 °C for 24 hours.

2. Preparation of BC Sheets

A rectangular container (30 cm×30 cm×3 cm) designed for the preparation of BC sheets was sterilized at 121 °C for 15 min. The basal medium was inoculated with 2% prepared cell culture as described in the literature [19] and incubated in static conditions at 30 °C for 15 days. The prepared BC sheets were treated with 500 mL of 0.3 M NaOH at 121 °C for 15 mints in order to disrupt and dissolve the microbial cells. The sheets were then washed thoroughly with distilled water until the pH of the water became neutral in order to remove the residual NaOH and culture medium components from the BC sheet. Thickness of the wet BC sheets was 2 mm. BC sheets were stored in the distilled water at 4 °C.

3. Preparation of BC-Ch Composites

Ch (medium molecular weight) was purchased from Aldrich (St. Louis, USA). BC-Ch composite were prepared by treating the BC sheets with Ch solution as introduced in the literature [11]. The BC sheets were immersed in the 1% acetic acid solution in which 1% Ch was dissolved. The BC sheets were treated with Ch solution for 5 hrs at different temperature of 0 °C, 25 °C, 50 °C and 75 °C to determine the effect of temperature on Ch penetration. The experiment was done separately in Static and Shaking (150 rpm) modes to check out the effect of the operation mode on the penetration of Ch inside the BC sheets. In the third experiment, the BC sheets were immersed in Ch solution and treated at 50 °C for 5, 20 and 40 hrs

in order to determine the effect of treatment time on the formation of the BC-Ch composite. Experiments were repeated several times. The chitosan solution on the surface of the BC-Ch composite sheets was removed with a filter paper and then, the BC-Ch composite sheet was freeze-dried at −40 °C.

4. Dry Weight Analysis

Dry weight analysis of the pure BC and BC-Ch composites produced in different experiments were done in order to determine the amount of Ch in the BC-Ch composite. A long sheet of wet BC was cut into small pieces. Surface water was removed and pieces of equal wet weight were taken. Some of these pieces were immersed in Ch solution for the synthesis of BC-Ch composites at different conditions, and the others were directly freeze-dried as pure BC (control). BC-Ch composite were also then freeze-dried. The dried weights of the pure BC and the BC-Ch composites were measured and compared to find out the amount of Ch present in BC-Ch composite. The amount of Ch present in the composite was then determined from dry weight analysis. Experiments were repeated several times to confirm the reproducibility.

5. FE-SEM Analysis

Scanning electron microscopy (SEM) of the freeze-dried BC and BC-Ch composite was done using a Hitachi S-4800 & EDX-350 (Horiba) FE-SEM (Tokyo Japan). Samples were fixed on a brass holder and coated with OsO_4 by VD HPC-ISW osmium coater (Tokyo Japan) prior to FE-SEM observation. FE-SEM analysis was used for determining the surface morphology and cross sectional views of the BC and BC-Ch composites.

6. Fourier Transforms Infrared Spectroscopy (FT-IR)

Infrared spectra of the dried BC and BC-Ch composite sheets were recorded with a Perklin Elmer FT-IR spectrophotometer (Spectrum GX & Autoimage, USA, Spectral range: 4,000-400 cm^{-1}; Beam spliter: Ge coated on KBr; Detector: DTGS; Resolution: 0.25 cm^{-1} (step selectable). For analysis, the samples were mixed with KBr pellets (IR grade, Merck, Germany). The obtained data was transferred to a PC to get the spectra.

7. X-ray Diffraction (XRD) Analysis

X-ray diffraction patterns of the samples were recorded on an X-ray diffractometer (X'Pert-APD PHILIPS, Netherland) with an X-ray generator: 3 KW and anode: LFF Cu. The radiation was Cu Kα of wavelength 1.54 Å. The X-ray generator tension and generator current was 40 kV and 30 mA, respectively. Angular scanning was varied 10-40°.

8. WHC and Water Retention Property

WHC of the samples was measured by the shake method [19]. Dried BC and BC-Ch sheets were placed in a water container for a sufficient time to completely swell up. These sheets were taken out from the storage container using tweezers. The samples were shaken twice quickly to remove the surface water and then, weighed. These samples were dried at room temperature for 48 h and their weights were measured at different time intervals. Samples were then dried for 12 h at 50 °C in order to completely remove the water from them. WHC was calculated as the mass of the water removed during drying per the dry weight of BC.

For determining the WRR the wet weights of the BC samples produced in static conditions were measured followed by continuously weighing the samples stored at ambient conditions at different time intervals until a constant dried weight was achieved. The

weight of the BC samples at different time intervals was plotted against time [19].

9. Mechanical Strength

The tensile properties of the BC and BC-Ch composite sheets were measured using an Instron Universal Testing machine (Model 4465, USA) according to the procedure of the American Society for Testing and Materials (ASTM D 882) [19]. Two metal clamps were placed at either ends of a 100 mm×10 mm rectangular strip of a dried BC sample in each case. The clamps were then mounted on an Instron 4465 that measured both elongation and maximum
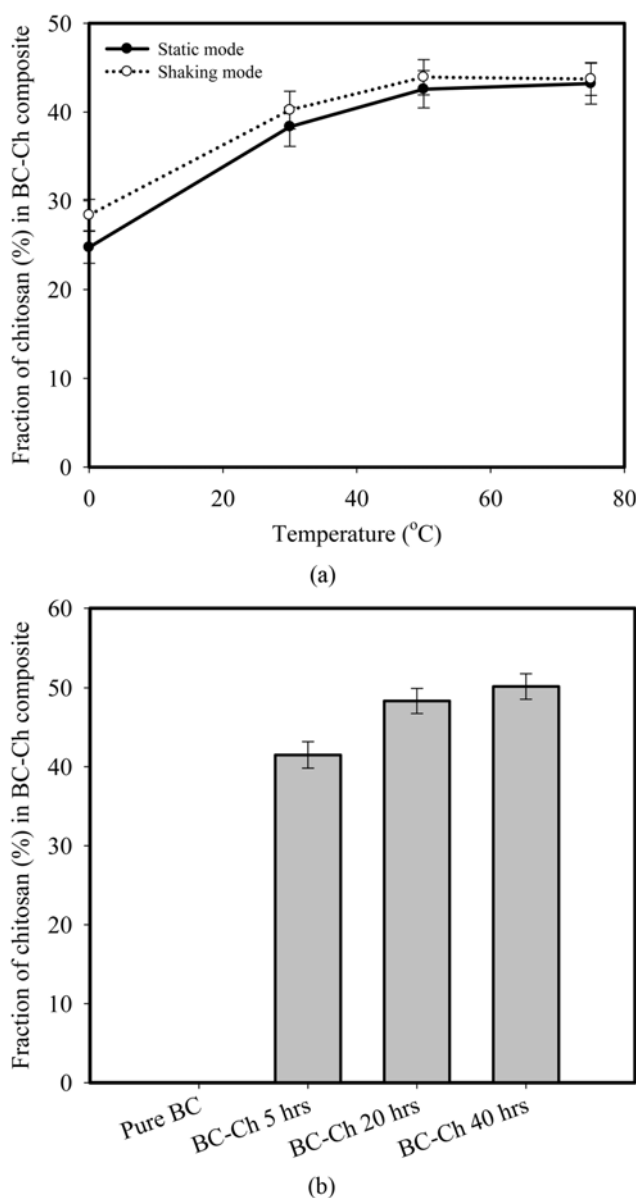

(a)

(b)

Fig. 1. (a) The amount of chitosan penetrated into the bacterial cellulose sheet. Bacterial cellulose sheet was dipped into 1% chitosan solution in the flask and remained for 5 hrs at various temperatures at the static and shaking mode of 150 rpm. (b) The increase in the amount of chitosan penetrated into the bacterial cellulose sheet with treatment time. Bacterial cellulose sheet was dipped into 1% chitosan solution and remained at 50 °C at shaking mode of 150 rpm.

tensile load before fracture. The experiment was repeated several times and the average values were determined.

RESULTS AND DISCUSSIONS

1. Dry Weight Analysis

BC was produced on the surface of the culture media as a gel like membrane under traditional static conditions. The thickness of the produced gel increased with culture time. The cellulose molecules are produced in the interior of the bacterial cell, which then expels out to produce the protofibril and then crystallize to form ribbon shaped microfibrils [20,21]. The dry weight analyses of the pure BC and BC-Ch composites were done in order to find out the amount of Ch in the BC-Ch composites. In each experiment, the dry weight of the composite was measured in order to find out the effect of varying the conditions on the degree of Ch penetration. The analysis done with the temperature variation (Fig. 1(a)) showed that the Ch penetration increased as the temperature increased from 0 °C to 50 °C. The reason may be that the Ch solution becomes thinner (less viscous) with increasing temperature, and can penetrate more easily. The effect was further analyzed up to 75 °C however, no significant increase in the Ch penetration was observed. The Ch penetration effect was also determined for two different operating modes, i.e., static and shaking. The repeated experiments done at different temperatures in both shaking and static conditions showed that Ch penetration was slightly higher in shaking conditions compared to static ones (Fig. 1(a)). The reason for the increased penetration may be the increase in the mass transfer coefficient due to the shaking. The third factor, which was studied for enhancing the Ch penetration inside the BC sheets, was the treatment time. The Ch penetration in BC sheets was checked with the treatment time at 50 °C and shaking mode of 150 rpm. The penetration of Ch greatly increased with treatment time up to 20 hrs and small increases were further found up to 40 hrs. The percentage of Ch reached more than 50% till 40 hrs of treatment (Fig. 1(b)). The increased presence of the Ch inside the BC-Ch composite was then further confirmed through structural analysis of the BC-Ch composite formed at 50 °C, in shaking mode of 150 rpm and varying treatment time of 5, 20 and 40 hrs.

2. FE-SEM Analyses of BC and BC-Ch Composites

Freeze-dried BC appears white in color and is very light. Pore size of the freeze-dried BC is larger than the air dried ones, because the surface tension effect caused by the liquid water destroys the pore structure during the vaporization process [22]. In freeze-dried BC, a direct conversion of the ice to vapors retains the pore geometry undisturbed. SEM analyses of the freeze-dried BC and BC-Ch composite were done to determine the morphology of the BC and also to verify the degree of penetration of Ch inside the BC sheets.

The SEM micrographs of the BC and BC-Ch composites, shown in Fig. 2(a) and 2(b) illustrate a three dimensional arrangements of the microfibrils. The microfibrils are randomly arranged in pure BC with a lot of empty space between them. Surface morphology of the pure BC can be easily differentiated from that of composites. There was a distinct covering layer on the surface of BC in BC-Ch composite as shown in SEM images. The Ch solution penetrates inside these empty spaces and generates strong interaction with the BC molecules [23]. Thus the surface of the BC sheets becomes more compact and denser with the treatment time. Higher resolution images
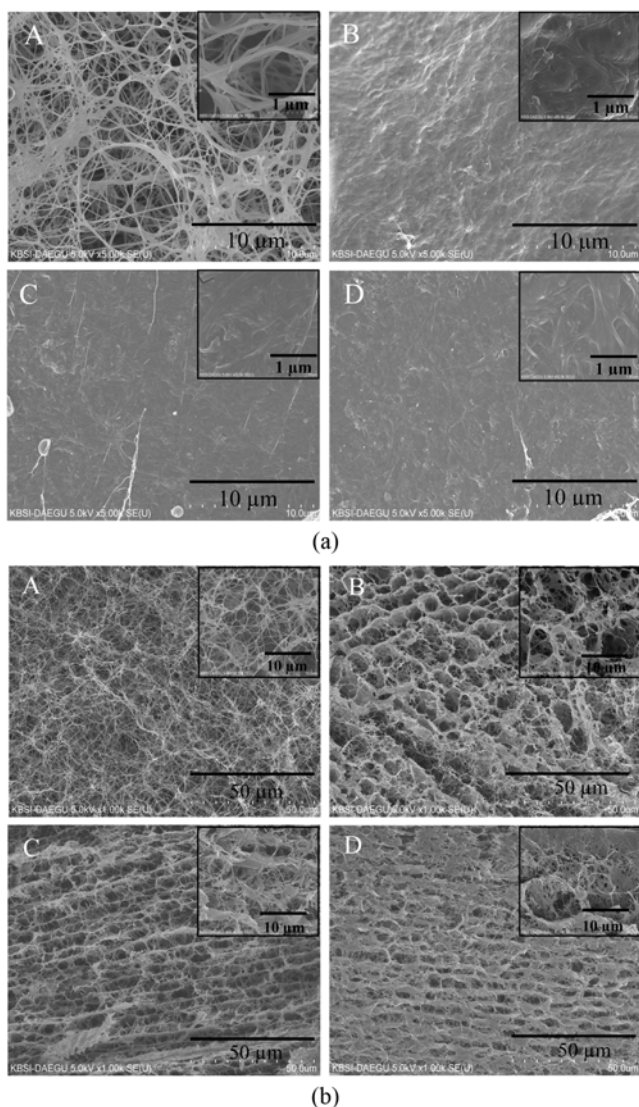
Fig. 2. (a) FE-SEM images of the surface morphology of pure BC (A), BC treated for 5 hrs (B), BC treated for 20 hrs (C) and BC treated for 40 hrs (D) in a 1% chitosan solution at 50 ºC and shaking mode of 150 rpm. (b) FE-SEM images of the cross section morphology of pure BC (A), BC treated for 5 hrs (B), BC treated for 20 hrs (C) and BC treated for 40 hrs (D) in a 1% chitosan solution at 50 ºC and shaking mode of 150 rpm.

further clarified the presence of a layer on the surface of the BC fibrils which had increased the thickness of the microfibrils. These increased thickness results in the increased mechanical properties of the composite materials [19,24]. The thickness of the microfibrils of the BC sheets was further increased with treatment time, which shows that the deposition and interaction of the Ch increases with the treatment time.

Cross sectional analysis of the BC and BC-Ch composite (Fig. 2(b)) shows that pure BC had a randomly distributed condensed network of microfibrils. Close image further clarified that the pure BC fibrils were thin, uncovered and had plenty of gaps between them. Cross sectional analyses of the BC-Ch composite on the other hand showed that the Ch not only formed layer on the BC surface

but also penetrated deeply inside the BC layer, and the structure of BC changed to an arranged network with specifically oriented pores. The penetration increased with treatment time and resulted in definite layers inside the polymeric BC sheets. As shown in Fig. 2(b), the cross sectional morphology of the BC-Ch (5 hrs) shows the presence of Ch inside the microfibrils and it had a specific shape in the fibrils arrangements. Furthermore, with increased time (20 hrs), the formation of definite layers occurred, which were even more concentrated with increased treatment time (40 hrs).

The FE-SEM analyses showed that the treatment of BC with Ch solution for longer time increased the penetration of Ch inside the BC sheets, which in turn increased the interaction between the BC and Ch molecules and thus would result in enhanced physico-mechanical properties.

3. FT-IR Analysis

FT-IR spectroscopy is an important technique to determine the functional groups and nature of a chemical bond in a molecule. The FT-IR analyses of the pure BC and the BC-Ch composites were done to verify the formation of the BC-Ch composites by analyzing the specific peak positions. The molecular structures of the cellulose and chitosan are very similar and the two polymers are miscible. Fig. 3 shows the spectra of the pure BC and BC-Ch composites. In case of pure BC, a broad characteristic peak for OH stretching appears at 3,332 cm^{-1} while for CH stretching a sharp peak appears at 2,896 cm^{-1} [25]. Another intense peak present at 1,651 cm^{-1} is assigned to the glucose carbonyl of cellulose [14]. Two more characteristic peaks at 1,427 cm^{-1} and 1,371 cm^{-1} show the symmetric deformation and bending vibration of CH respectively [25]. The peak for C-O-C stretching appears at 1,035 cm^{-1}. The spectral analyses of the BC-Ch composites show the presence of about all the characterized peaks of BC, however, there are some additional peaks, which justify the presence of the chitosan fragments. A very broad peak at ~3,325 in the composite spectra shows the presence of hydrogen bonded OH stretching, while the NH peaks which also appears in the same range has also combined with it [11,25]. The increased hydrogen bonding of the peaks shifted the peak towards
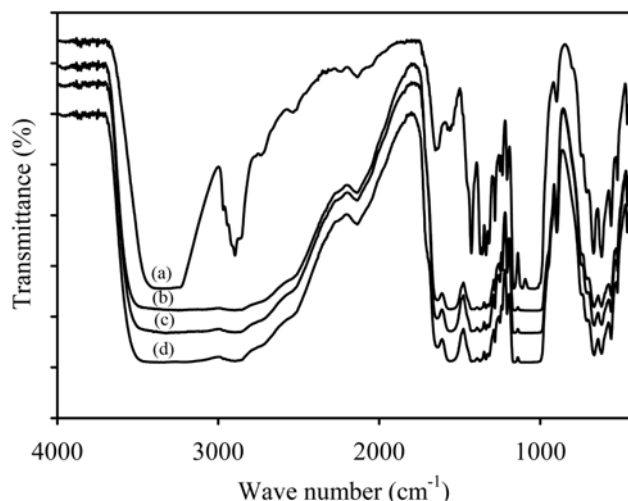

Fig. 3. FT-IR spectrum of pure BC (a), BC treated for 5 hrs (b), BC treated for 20 hrs (c) and BC treated for 40 hrs (d) in a 1% chitosan solution at 50 ºC and shaking mode of 150 rpm.

Table 1. Assignment of FT-IR spectra of pure BC and BC-Ch composite prepared by the treatment for a different treatment time in a 1% chitosan solution at 50 °C and shaking mode of 150 rpm

	BC, BC-Ch composite characteristics peaks positions			
Functional group	BC (Control)	BC-Ch composite (5 hrs)	BC-Ch composite (20 hrs)	BC-Ch composite (40 hrs)
	Wave number (cm^{-1})	Wave number (cm^{-1})	Wave number (cm^{-1})	Wave number (cm^{-1})
O-H / N-H	3332.20	3325.00	3324.82	3324.64
C-H stretching	2896.20	2896.13	2896.18	2896.24
CO/ NCO	1651.68	1643.01	1639.44	1639.41
NH$_2$ deformation	-----	1555.08	1554.60	1551.08
CH asymmetric deformation	1427.99	1420.24	1420.05	1420.02
CH bending	1371.61	1374.41	1374.80	1374.89
C-N stretching (primary amides)	------	1405.12	1405.12	1405.12

lower wavelength compared to pure BC [25]. As shown in Table 1, peak for carbonylic (CO) group appears at 1,643.01 cm^{-1} for the composite prepared with 5 hrs of treatment and at 1,639.60 cm^{-1} and 1,639.41 cm^{-1} for the composites prepared with 20 and 40 hrs of treatment. This decrease in the CO stretching frequency compared to pure BC (1651) is due to the increased hydrogen bonding present in the composites [25]. Furthermore, the shift towards a lower wave number region shows that the interaction has been strengthened between the BC and Ch molecules. A dominant characteristic peak appeared at about 1,555, 1,554 and 1,551 cm^{-1} for the BC-Ch composites prepared with 5, 20 and 40 hrs of treatment, corresponds to NH$_2$ deformation vibration [11,25]. This also shows the presence of the chitosan segments in the composite. A new small peak found at about 1,405 cm^{-1} in all composite was assigned to the C-N stretching of the primary amides. The appearance of broad peak in the composite spectra and also some additional peaks related to the CN and NH suggest the presence of a chitosan molecule bonded to the BC through intermolecular hydrogen bonding interactions. Thus the FT-IR study greatly supports the surface and cross sectional morphology of the composite determined through FE-SEM analyses.

4. XRD Analysis

The broad diffraction peaks are usually observed for BC [14] because BC is not a complete crystalline material. Crystallinity and strength of hydrogen bonding can bring about morphological changes in BC microstructures [19]. X-ray diffraction analyses were required in order to investigate the micro structural changes in the BC sheets caused by the penetration of Ch.

The XRD patterns of BC and BC-Ch composite films are shown in Fig. 4. All the patterns show three distinct peaks which appears at 2-theta 14.2°, 16.6°, and 22.4° and arises from planes <-110>, <110>, and <200>, respectively. An amorphous halo is observed at 2-theta 19.56. As a BC polymer cannot be 100% crystalline, this amorphous peak intensity varies from sample to sample and a well-developed method is usually used in order to investigate the crystallinity index for cellulose [26]. The intensity values at 2-theta 19.56 (amorphous peak) and at 2-theta 22.4, determined after baseline correction were incorporated into the (CrI) eq. for estimation of the crystallinity index. The equation is given as;

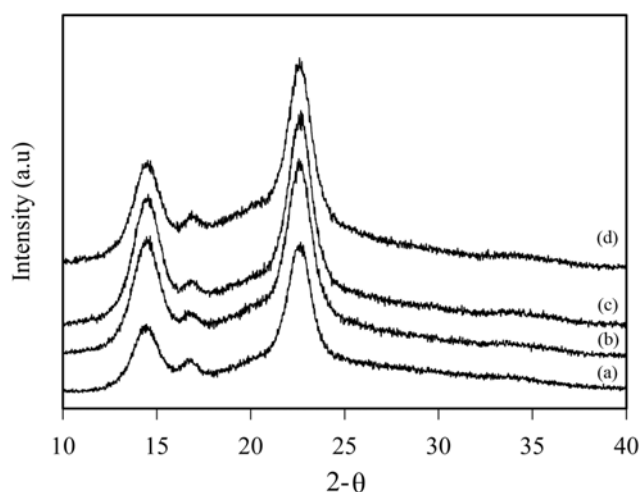$$CrI=[\{I\,(200)-I\,(am)/I\,(200)\}\times100]$$

Fig. 4. XRD pattern of pure BC (a), BC treated for 5 hrs (b), BC treated for 20 hrs (c) and BC treated for 40 hrs (d) in a 1% chitosan solution at 50 °C and shaking mode of 150 rpm.

Where CrI is the crystallinity index, I (200) is the peak intensity of (200) plane and I (am) is the intensity of the amorphous peak.

Table 2 shows that the crystalline indices of the composite BC were somewhat different from the pure BC. The crystallinity has been decreased in BC-Ch composite compared to pure BC. The relative crystallinity of the pure BC was 71.7%, which decreased to 65% in BC-Ch (5 hrs) composites and further decreased slightly to 64.5% and 64.3% in BC-Ch composite formed with 20 and 40 hrs of treatment, respectively. This is in accordance with some previous studies which showed that the additives usually resulted in decreased crystallinity [11,14,27]. The decrease in crystallinity is due to the incorporation of Ch molecules in the BC microfibrils. The penetrated Ch molecules interact with the cellulose chains through hydrogen bonding interactions. This results in the disturbance of the original hydrogen bonding between the BC microcrystalline chains, and ultimately results in a slightly lower crystallinity of the composites.

Cellulose usually consists of various compositions of two allomorphs, alpha cellulose (Iα) and beta cellulose (Iβ) depending upon its source. Iα is the hard part of cellulosic material that is insoluble in 17.5% alkaline solution at 20 °C temperature, Iβ cellulose is dis-

Table 2. d-spacing, crystallite size, cellulose Iα, Iβ and Iγ content and Crystallinity index of BC and BC-Ch composite determined through X-ray diffractograms

Sample	d-Spacing (Å)			Crystallite sizes (nm)			Cellulose type (%)			Crystallinity index (%)
	d1	d2	d3	Crystal 1	Crystal 2	Crystal 3	Iα	Iβ	Iγ	
BC (Control)	6.124	5.305	3.936	4.8	5.8	5.7	29.75	57.52	13.03	71.7
BC-Ch (5hrs)	6.130	5.288	3.925	4.6	7.7	5.4	39.53	56.34	4.12	65.0
BC-Ch (20 hrs)	6.115	5.272	3.920	4.7	8.4	5.5	39.82	56.63	3.55	64.5
BC-Ch (40 hrs)	6.126	5.261	3.920	4.7	9.1	5.4	36.47	59.96	2.60	64.3

solved under same conditions and then pricipitate if alkaline solution is acidified with acids. Iα and Iβ cellulose contain triclinic and monoclinic crystal planes, respectively and the peaks appearing in the XRD spectra are governed by the diffraction of triclinic and monoclinic crystal planes [27]. Both Iα and Iβ were primarily observed in all the samples of pure and composite BC, which were characterized by the diffractions from the triclinic and monoclinic planes. However, besides the two specific types, one other type called gamma cellulose (Iγ) is also present. Iγ is part of cellulosic material that is also soluble under above alkaline solution and does not precipitate but dissolves when treated with acids. Iγ is actually the composite of triclinic and monoclinic diffractions. The fraction of triclinic in Iγ cellulose is larger than that of monoclinic as clear from the d-spacing values in Table 2 because the interplanar distance of the triclinic (200) plane is closer to that of Iγ than the monoclinic (110) plane [27]. An interesting phenomenon observed here is the decrease in the percentage of Iγ with incorporation of Ch in the BC sheets. The penetration of Ch in the BC sheets could possibly disturb the symmetry of a particular plane. This can be further confirmed from the increased percentage of the Iα cellulose in the BC-Ch composites, which is evidence of the transformation of the Iγ cellulose to the Iα cellulose.

The crystallization of cellulose is the major rate-limiting step in its synthesis by microbes [28]. The initial addition of some additives during the production of BC can disturb the crystallization process and thus can affect the production. However, very small modifications in the crystalline size of the BC may be observed when pure BC is treated with some additives substances. In the present study, the size of crystal 2 in the BC-Ch composites increased showing a decrease and broadening of the 2nd peak. However the d-spacing remained almost the same in the pure BC and in all the types of composites as shown in Table 2.

The comparative results of the composite formed at different time intervals showed that the crystallinity decreased to a small extent over the treatment time. This also showed that the increased Ch penetration had some effect on the nature of hydrogen bonding between Ch and BC molecules.

5. Water Holding Capacity and Water Release Rate

Keeping the wound moist is an important factor in the modern treatment of wounds and the healing process. The reason is that a moist environment increases the penetration of the active substances. Moreover the dressing change does not harm the newly produced skin [19,29]. Due to its hydrophilic nature, high water absorbing capability, and slow water release rate, BC has been extensively used as a wound dressing materials. In the present work, WHC of the pure BC and BC-Ch composite were determined through re-
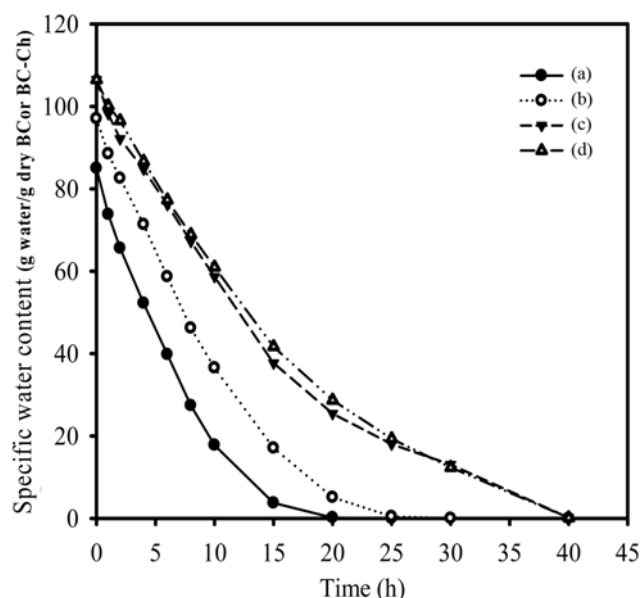

Fig. 5. Water holding capacity and Water release of pure BC (a), and BC-Ch composites prepared by the treatment for 5 (b), 20 (c) and 40 hrs (d) in a 1% chitosan solution at 50 ºC and shaking mode of 150 rpm.

peated experiments. The results show (Fig. 5) that the pure BC absorbed 85 times its dry weight in water. However, the composites showed a much higher WHC than the pure BC. The WHC increased with Ch penetration in the BC matrix. The WHC of the BC-Ch composite prepared with 5 hrs of treatment was 97 times and the WHC of the BC-Ch composite prepared with 20 and 40 hrs of treatment was 106 times its dry weight in water. The results can be explained by the hydrophilic nature of Ch. The incorporation of Ch inside the cellulose network results in the enhanced water absorption [14]. The increase in Ch incorporation over the treatment time shown in the SEM analysis increased the WHC of the BC-Ch composite. The increased penetration of Ch results in better hydrogen bonding interaction between the BC and Ch molecules, which is also a factor causing an increase in the water holding capacity [30]. Nearly the same WHC for the BC-Ch composites prepared with 20 or 40 hrs of treatment indicates that the BC was almost saturated with Ch in 20 hrs of treatment.

WRR of the BC and BC-Ch composites was also observed to determine the validity of the BC-Ch composite. The results shown in Fig. 5 indicate that WRR of the BC-Ch composites was much lower than that of the pure BC. The water retention properties of
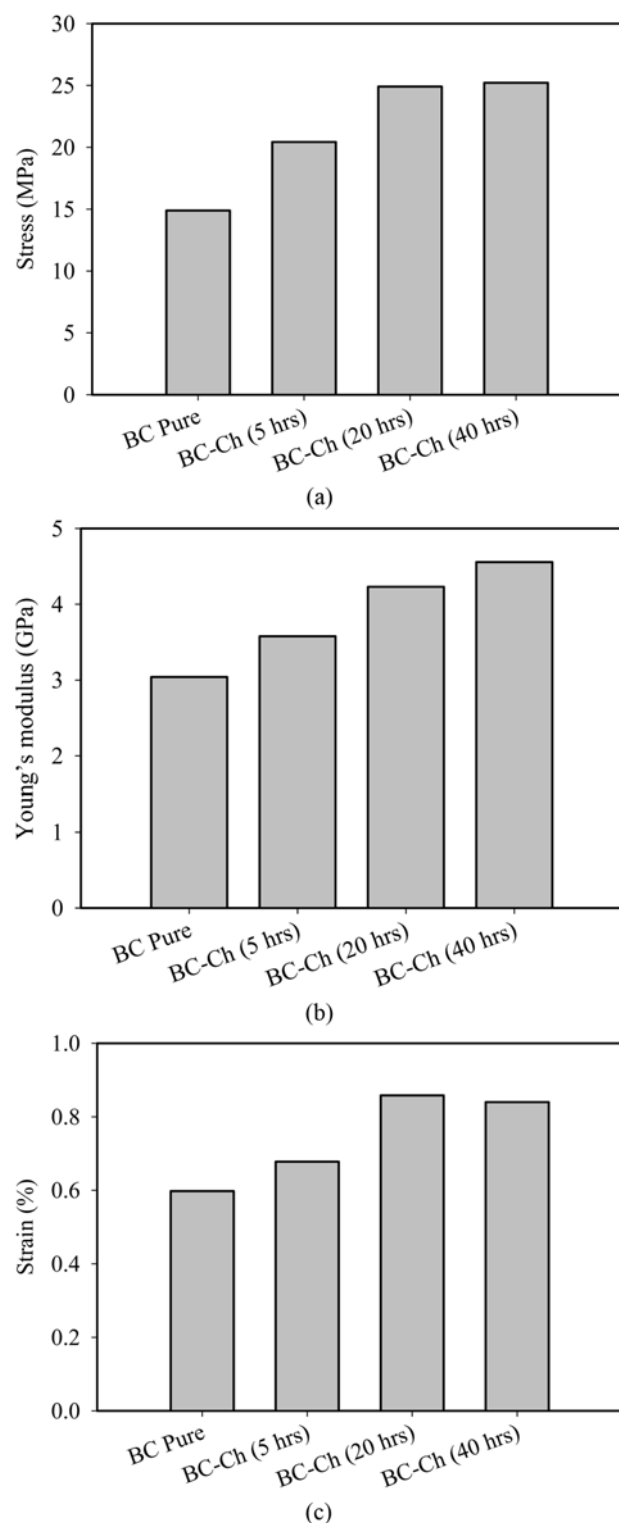
(a)

(b)

(c)

Fig. 6. (a) The tensile strength at break point of pure BC and BC-Ch composites, prepared by the treatment for 5, 20 and 40 hrs in a 1% chitosan solution at 50 ℃ and shaking mode of 150 rpm. (b) The Young's modulus of pure BC and BC-Ch composites prepared by the treatment for 5, 20 and 40 hrs in a 1% chitosan solution at 50 ℃ and shaking mode of 150 rpm. (c. The elongation (% strain) at the break point of pure BC and BC-Ch composites prepared by the treatment for 5, 20 and 40 hrs in a 1% chitosan solution at a shaking mode of 150 rpm.

the BC-Ch composite increased over the treatment time. The absorbed water in the pure BC was almost released in 20 hrs and a constant dry weight was achieved after 25 hrs at room temperature. The BC-Ch composites prepared with 5 hrs of treatment retained the water till 30 hours and the BC-Ch composites prepared with 20 and 40 hrs of treatment released water slowly till 40 hrs. The decrease in the release rate of the composites is due to the slow passage through the interfibril wall inside the BC-Ch matrix and on the sheet surface [14]. As shown in the SEM micrographs, the Ch forms a covering layer on the surface of the BC sheets, which retards the escape of the water molecules through the BC surface.

The high values of WHC and slow WRR obtained for the BC-Ch composite are important. These BC-Ch composites can be applied in biomedical fields especially in wound treatments because the BC sheets are used as dressing materials, and chitosan has excellent healing capabilities. The combination of both properties in a single composite material will really be helpful in rapid wound healing. Therefore, the higher WHC and slower WRR values achieved in the composite is another advantage of BC-Ch composite materials to enhance its biomedical applicability.

6. Mechanical Strength Analysis

Mechanical property is a major factor for BC to be used as a scaffold material and in biomedical fields like wound dressing materials, tissue engineering, etc. [31]. A tensile test was done to investigate the intrinsic mechanical properties of the BC and to investigate the effect of the Ch penetration on the strength of the composite formed. The analyses were done repeatedly for each sample and then the average values were calculated. Fig. 6(a), (b), and (c) show the analysis of the tensile strength for the BC and BC-Ch composite formed. The maximum tensile strength values at the break point of the composite were higher than the pure BC. Compared to the pure BC, the maximum values of the tensile stress were about 1.4 times higher for the BC-Ch (5 hrs) composite and about 1.7 times higher for the BC-Ch composites prepared with 20 and 40 hrs of treatment. Similarly, the Young's modulus values also increased for the composite compared to the pure BC. The Young's modulus of the pure BC was 3.04 GPa which increased to 3.58 GPa for the BC-Ch composite prepared with 5 hrs of treatment. The Young's modulus increased to 4.23 GPa and 4.56 GPa for the BC-Ch composites prepared with 20 and 40 hrs of treatment, respectively. Moreover, the (%) strain values of the BC-Ch composite were slightly higher compared to the pure BC. The results obtained from the mechanical strength analysis highly support the other experimental data and is in accordance with previous studies. As shown in the SEM micrographs, the Ch deeply penetrated inside the BC sheets and formed a dense network inside the microfibrils of the BC. When a pull force is applied on the BC fibrils in BC-Ch composites, the dense Ch network shares the applied tension, and thus, composite withstand a stronger pull force than the pure BC fibers alone. Moreover, Ch forms a coating on the BC fibrils, which increases its thickness and the ultimate results is its high mechanical strength [24]. The results also show that the mechanical strength of the composite increased with additional treatment time.

CONCLUSION

The penetration of Ch inside the microfibrils network of BC re-

sulted in BC-Ch composite having highly enhanced physic-mechanical properties. Higher penetration was observed at 50 °C temperature in shaking operation mode. The fibrils became much thicker with deep penetration of Ch in the BC matrix. The dense network of Ch inside the BC sheets reinforced the BC microfibrils and thus enhanced its mechanical strength. The WHC of the composite material was much higher than the pure BC because the hydrophilic groups of Ch also facilitate water adsorption. Moreover, the WRR of the BC-Ch composite was much lower than the pure BC making BC-Ch composites a valuable scaffold material in wound treatment.

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