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Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose

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ABSTRACT

The addition of certain supplementary carbon sources to the culture media can influence the production, structural features and mechanical properties of bacterial cellulose (BC). In this study, different concentrations (0, 1, 2 and 4%) of a by-product, single sugar α-linked glucuronic acid-based oligosaccharide (SSGO), were added to the culture media during the production of BC. Production with 1% (BC1), 2% (BC2) and 4% (BC3) SSGO led to increases in BC production of 10.45, 12.74 and 9.01 g/L, respectively, after 10 days of cultivation under static conditions, while it was only 7.4 g/L when no SSGO was added (BC0). The structures of BC0, BC1, BC2, and BC3 were confirmed by XRD and FT-IR analysis. FE-SEM micrographs showed increased fibril thickness and decreased pore size in the SSGO added samples. The tensile strength of the BC0 was 16.73 MPa, while it was 25.05 MPa for BC1. However, with further increases in the concentration of SSGO, the tensile strength decreased to 20.76 and 19.77 MPa for BC2 and BC3, respectively. The results of this study provide further insight into the additive role of SSGO and improvement of the physico-mechanical properties of BC.

1. Introduction

Bacterial cellulose (BC) is a pure form of cellulose that is free from impurities such as lignin and hemicelluloses. Its structural features and extra purity bestow BC with several advantages over plant cellulose. The ultra-fine compact fiber network structure and tensile strength of BC makes it an extra crystalline material with superior mechanical properties (Backdahl et al., 2006; Phisalaphong, Suwanmajo, & Tammarate, 2008). BC has also high water holding capacity, hydrophilicity, biocompatibility, polyfunctionality, and the ability to be molded into three-dimensional (3D) structures during synthesis (Backdahl et al., 2006; Czaja, Krystynowicz, Bielecki, & Brown, 2006; Phisalaphong et al., 2008). BC is considered an attractive biomaterial due to its potential for widespread applications in the food, paper, acoustic membrane, and pharmaceutical industries (Phisalaphong et al., 2008). BC has been used in drug delivery systems and enzyme immobilization and as a conductive material for various applications (Ciechanska, 2004; Wu & Lia, 2008; Yoon, Jin, Kook, & Pyun, 2006). Additionally, the never dried BC gels have high strength and are important in the biomedical field as wound dressing materials (Ciechanska, 2004; Czaja et al., 2006). BC has also been shown to play a pleiotropic role in artificial skin, artificial blood vessels, scaffolds for tissue engineering, treatment for skin injuries and severe body burns (Czaja et al., 2006), medical pads and dental implants (Czaja, Young, Kawechi, & Brown, 2007; Klemn, Schumann, Udhardt, & Marsch, 2001; Wan, Hutter, Millon, & Guhados, 2006).

Several attempts have been made to enable greater production of BC at a reasonable cost for commercial application. These include exploration of inexpensive sources, optimization of culture conditions, design of various bioreactors, and the addition of supplementary materials to the growth media (Bae, Sugano, & Shoda, 2004; Keshk, 2006; Kurosumi, Sasaki, Yamashita, & Nakamora, 2009; Park, Jung, & Park, 2003; Shah, Ha, & Park, 2010). One of the major causes contributing to the low BC yield is the generation of side products such as water soluble oligosaccharides (Khan, Khan, & Park, 2008). Accordingly, any strategy adopted to inhibit the production of such by-products could significantly enhance the BC yield. In our previous investigations, the addition of 1% water-soluble oligosaccharide (a single sugar α-linked glucuronic acid-based oligosaccharide (SSGO)) to the culture medium was found to enhance the production of BC (Ha, Shah, Ul-Islam, Khan, & Park, 2011). Additionally, a metabolic pathway was proposed which revealed that the formation of by-products could be blocked by their initial addition to the growth media (Ha et al., 2011).

Several studies have shown that the culture medium composition and fermentation conditions significantly affect the chemical structure and composition of microbial polysaccharides, including

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BC (Duta, França, & Lopes, 2006; Watanabe et al., 1998). The mechanical properties of BC depend on the fibril arrangements and density, which are affected by various factors including culture time, culture conditions, carbon source, inoculum amount, treatment and drying method (Guo & Catchmark, 2012; Tang, Jia, Jia, & Yang, 2010). In the presence of supplementary carbon sources, the production of BC occurs for relatively longer times, resulting in high micro fibril secretion and stronger BC fibers (Ul-Islam, Khan, & Park, 2012a). The relatively thick and dense fiber can ultimately modulate the mechanical properties of BC. The addition of 1% SSGO to the culture media was shown to produce higher BC with prolonged production time (Ha et al., 2011). However, further investigation of the effect of different SSGO concentrations on the production, structural variation, and mechanical properties of the formed BC is needed. In the present study, BC was produced with 0, 1, 2, and 4% SSGO added to the growth medium and the effects of the different SSGO concentrations on the relative production, structure variation and mechanical properties of BC were evaluated. The results of this study will provide further insight into the additive role of SSGO and enhanced physico-mechanical properties of BC.

2. Materials and methods

2.1. Microorganism and cell culture

The basal medium (MAE) was prepared by dissolving 10 g of glucose, 10 g of yeast extract, 7 g of peptone, 1.5 mL of acetic acid, and 0.2 g of succinate in 1 L of distilled water. The agar plates used to culture *Gluconacetobacter hansenii* PJK (KCTC 10505BP) were prepared from basal medium with 20 g agar/L. The pH of the medium was adjusted to 5.0 with 1 N NaOH, after which it was autoclaved for 15 min at 121 °C. *G. hansenii* PJK colonies were then inoculated into 50 mL medium in a 250 mL flask and incubated at 30 °C for 24 h while shaking at 150 rpm.

2.2. SSGO and BC production

The SSGO was produced according to a previously described method (Khan et al., 2008). Briefly, BC0 was produced in a static culture by inoculating 5.0% of the *G. hansenii* PJK broth into MAE medium at 30 °C at pH 5. BC1, BC2, and BC3 were produced by adding 10, 20 and 30 g SSGO/L to the basal media, respectively, before adjusting the pH. The rest of the process was the same as that for BC0. The produced BC was then harvested and treated with 0.3 N NaOH to remove the cells and debris, after which the BC sheets were washed thoroughly with deionized water until the pH became neutral and then freeze dried until used for various analyses.

2.3. Field emission scanning electron microscopy (FE-SEM)

Scanning electron microscopy (SEM) of the freeze-dried samples was performed using a Hitachi S-4800 & EDX-350 (Horiba) FE-SEM (Tokyo Japan). Samples were fixed onto a brass holder and coated with osmium tetra oxide (OsO_4) using a VD HPC-ISW osmium coater (Tokyo Japan) prior to FE-SEM observation.

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of all of the BC samples were recorded using a Perkin Elmer FT-IR spectrophotometer (Spectrum GX & Autoimage, USA, Spectral range: 4000–400 cm^{-1}; beam splitter: Ge coated on KBr; detector: DTGS; resolution: 0.25 cm^{-1} (step selectable)). For analysis, the samples were mixed with KBr (IR grade, Merck, Germany) pellets and processed further to obtain IR data, which were transferred to the PC to acquire the spectra.

2.5. X-ray diffraction (XRD) analysis

XRD patterns of the samples were recorded using an X-ray diffractometer (X'Pert-APD Philips, Netherlands) with an X-ray generator (3 kW) and anode (LFF Cu). The radiation was Cu Kα at 1.54 Å, the X-ray generator tension and current was 40 kV and 30 mA, respectively, and the angle of scanning was varied from 5° to 80°. The crystallinity indices of the BC samples were determined from the integrated areas of the crystalline and amorphous phases, as reported previously (Focher et al., 2001).

2.6. Mechanical properties

The tensile properties of the BC0, BC1, BC2 and BC3 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) (Shezad, Khan, Khan, & Park, 2010). Two metal clamps were placed at either end of each 100 mm × 10 mm rectangular strip of dried sample. The clamps were then mounted on an Instron 4465 that measured both elongation and maximum tensile load before fracture. The experiment was repeated several times and the average values were taken.

3. Results and discussion

Various additives can significantly affect the production of BC by either enhancing the activities of the producing microorganisms or providing a supplementary nutritional source. The underlying mechanisms responsible for this enhancement may include variations in the viscosity, pH, and oxygen transfer rate of the media (Chao, Mitarai, Sungano, & Shoda, 2001). Two major factors influencing the production of BC are the quantity of the nutritional source (cellulose producing carbon sources) and the relative by-product formation (Ha et al., 2011). Various carbon sources such as glucose, fructose, sucrose, and ethanol are utilized in the production of BC (Çoban & Biyik, 2011), and its production decreases after complete consumption of these primary carbon sources. The presence of additives can supplement the primary carbon source and can keep up the BC production provided live cells are present in the media. Moreover, this strategy can inhibit the by-product formation, further enhancing BC production (Ha et al., 2011). SSGO is a by-product which is produced during the BC production by *G. hansenii* PJK and its addition to the culture medium can enhance the BC production by supplementing the primary carbon source and by inhibiting self-production (Ha et al., 2011). Therefore, the effects of various concentrations of SSGO on the production and productivity of BC, structural variations, and mechanical properties were investigated.

3.1. Effect of SSGO concentration on BC production

The relative production of BC0, BC1, BC2, and BC3 is shown in Fig. 1. The results indicate positive impacts of SSGO addition on BC production. Specifically, BC production increased significantly with the addition SSGO, from 7.4 g/L in BC0 to 10.45 and 12.74 g/L for BC1 and BC2, respectively, on tenth day of cultivation. However, a further increase in SSGO concentration to 4% did not have any significant effect on BC3 production. The total production of BC3 on the tenth day of cultivation was 9.01 g/L. Taken together; these results showed that the addition of 2% SSGO is the optimum quantity for the production of BC. As mentioned above, SSGO is produced as a by-product during BC production (Khan et al., 2008). The present study and our previous investigation (Ha et al., 2011) were based on the principle of blocking the production of side products by adding SSGO to the media so it could be utilized as a secondary carbon source for BC production. Accordingly, the amount of SSGO

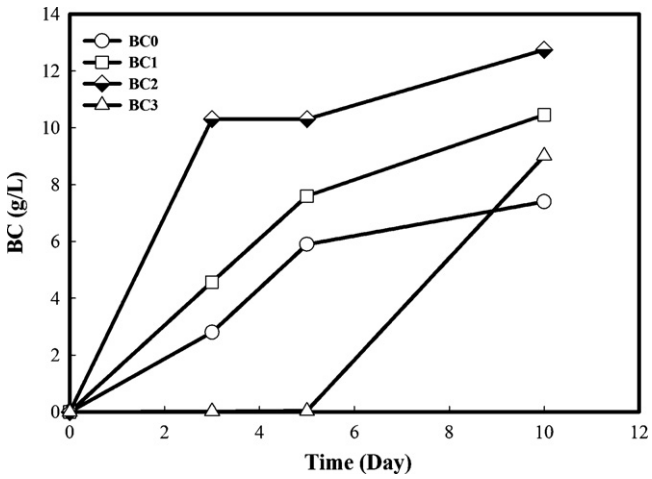

Fig. 1. Time course of BC production by *G. hansenii* PJK in MAE medium containing 0, 1, 2 and 4% SSGO during static culture at 30 °C.

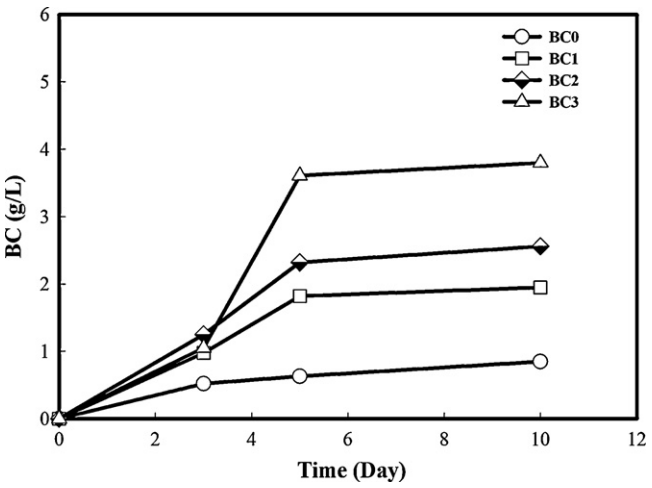

Fig. 3. Time course of BC production by *G. hansenii* PJK in MAE medium containing 0, 1, 2 and 4% SSGO instead of glucose during static culture at 30 °C.

was monitored to evaluate its production or consumption during BC production. The relative amounts of SSGO produced during the production of BC0, BC1, BC2 and BC3 are shown in Fig. 2. In MAE media, a certain amount of SSGO was produced during BC production, whereas in SSGO amended media, the production of SSGO was stopped and the amount of SSGO present at the end of the cultivation period was decreased. The final amount of SSGO measured on the tenth day of cultivation was 1.8, 6.07, 15.6 and 30.4 g/L in BC0, BC1, BC2 and BC3, respectively. The increase in BC production and relative decrease in SSGO concentration demonstrated the consumption of SSGO during BC production and its utilization as a supplementary carbon source, as in our previous studies (Ha et al., 2011). As shown in Fig. 1, the productivity of BC was also affected by the presence of SSGO in the media. The BC production increased immediately in response to the addition of 1% and 2% SSGO to the media, showing that the activity of the bacterial cells was positively affected by the presence of SSGO in the media. The dissolution of SSGO in the media increased its viscosity, which enhanced the cell activity and oxygen uptake as reported previously (Ishida, Sugano, Nakai, & Shoda, 2002). However, further increases in SSGO (4%) increased the viscosity to a very high level. This highly viscous medium might retard the growth, movement, and activity of bacterial cells. This speculation is well supported by the very low BC3 production that was observed until the fifth day and followed by a rapid increase to 9.01 g/L on day 10. The results also showed that the SSGO was produced in the beginning of the cultivation period, and then decreased until finally reaching 30.4 g/L (Fig. 2). These results showed that the glucose was utilized by the cells for their growth, after which BC was produced at the expense of SSGO (Fig. 2).

Another set of experiments was conducted to visualize the production of BC in the MAE media without glucose. As shown in Fig. 3, the maximum production obtained after 10 days was 0.85, 1.95, 2.56, and 3.80 g/L for BC0, BC1, BC2 and BC3, respectively. These findings clearly demonstrate that SSGO has been utilized in the production of BC. The highest BC production occurred with the 4% SSGO media. As shown previously (Ha et al., 2011), SSGO is a by-product produced from glucose during the production of BC. However, the synthetic pathway shows that the production of SSGO is a bidirectional process (Ha et al., 2011), and it is possible that a certain amount of SSGO is converted to glucose or UDP-glucose, which is in turn utilized in the production of BC. Fig. 4 indicates the consumption of SSGO that utilized for BC production. With the results of Figs. 3 and 4, the BC production increased with the consumption of SSGO and the maximum BC was achieved when initial SSGO was 4%.

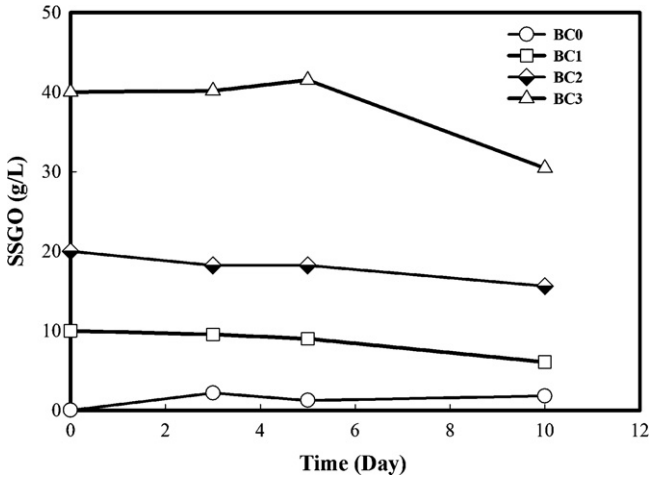

Fig. 2. SSGO concentration profile during BC synthesis by *G. hansenii* PJK in MAE media containing 0, 1, 2, and 4% SSGO under static culture at 30 °C.

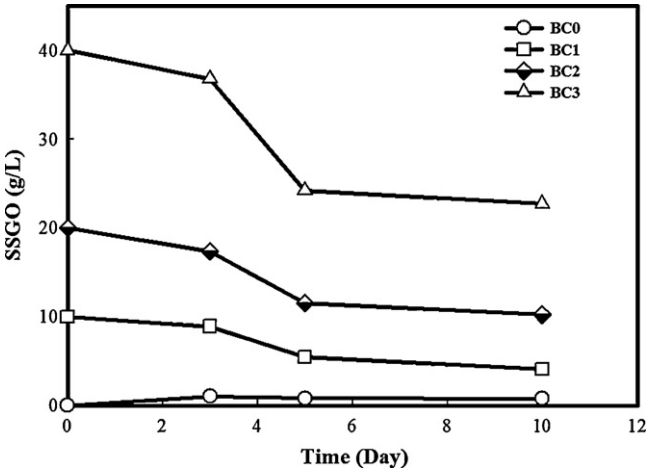

Fig. 4. SSGO concentration profile during BC synthesis by *G. hansenii* PJK in MAE media (instead of glucose) containing initially 0, 1, 2, and 4% SSGO under static culture at 30 °C.

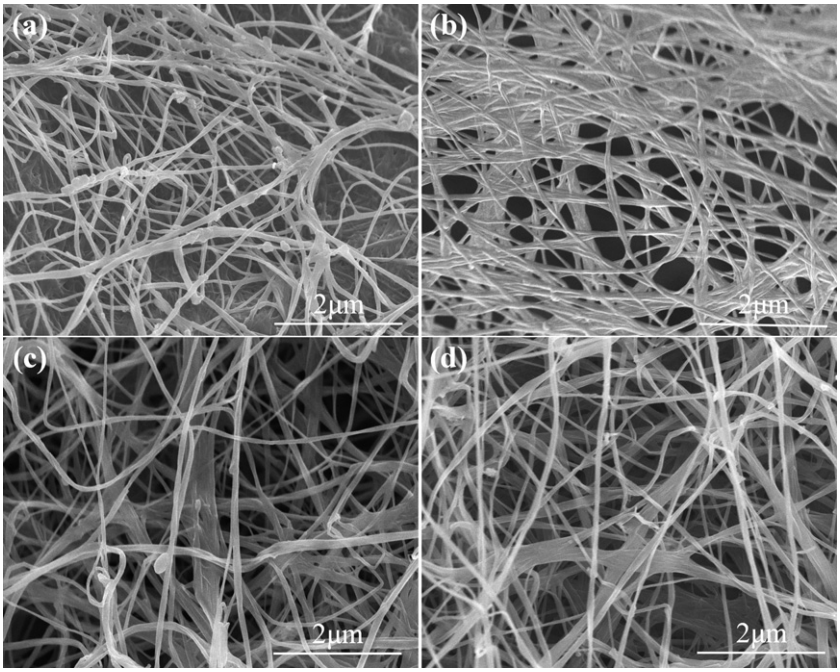

Fig. 5. FE-SEM analysis showing the morphology and arrangement of cellulose fibrils of BC0 (a), BC1 (b), BC2 (c) and BC3 (d). The BC (all) samples were produced by cultivation under static conditions at 30 °C for 10 days.

3.2. Structural morphology through FE-SEM analysis

Flat BC sheets produced on the surface of culture media consist of densely arranged three-dimensional web shaped fibrils. The mechanical properties of the BC sheets are highly dependent on the thickness and arrangement of these fibrils (Ul-Islam et al., 2012a). FE-SEM analyses of BC0, BC1, BC2 and BC3 were carried out to identify structural mutations in the BC that occurred during its synthesis in the presence of various quantities of SSGO in the media.

The FE-SEM analysis results of all BC samples are presented in Fig. 5. A reticulated fibril arrangement with definite variation in their size and density can be clearly observed from these SEM micrographs. It was obvious that increasing the SSGO contents in the media had a positive effect on the thickness, density and compactness of the BC micro-fibrils. The effects of culture conditions, treatment time, amount of inoculum, carbon source, treatment and drying method on the fibril thickness and density have previously been reported (Guo & Catchmark, 2012; Tang et al., 2010). The cellulose fibrils extruded from the microorganisms aggregate and become thick and compact as per their production (Horii et al., 1997). The presence of carbon sources in the media results in the secretion of more fibrils with the passage of time and can enhance the thickness and density of fibrils (Tang et al., 2010). SSGO added to the culture media supplements the primary carbon source (glucose) for BC production (Ha et al., 2011), resulting in the production continuing after the complete consumption of glucose. The production of more fibrils results in thick and dense BC fibrils that improve the physico-mechanical properties of the BC (Shezad et al., 2010). The nature of the carbon source can cause structural variations in BC due to the production of side products that may change the culture conditions, including the pH (Shezad et al., 2010; Tang et al., 2010). The addition of SSGO to the culture medium at the beginning of fermentation inhibits its self-production (Ha et al., 2011), which can ultimately affect the culture conditions and lead to variations in the physical structure of the final product. Variations in the structural characteristic of the BC with the addition of additives such as agar, carboxymethyl cellulose, microcrystalline cellulose, and sodium alginate have already been reported (Cheng, Catchmark, & Demirci, 2009). The results of the present study also demonstrated that the addition of various concentrations of SSGO causes modifications of the size and arrangement of fibrils that will affect the mechanical properties of the BC sheets.

3.3. X-ray diffraction (XRD) analysis

The structural variations induced in crystalline materials such as BC during synthesis by varying the media constituents can be monitored by XRD. In the present study, XRD analyses were carried out to investigate the micro-structural changes in BC sheets caused by the presence of various quantities of SSGO in the media. The XRD patterns of BC0, BC1, BC2 and BC3 are shown in Fig. 6. All of the patterns show three distinct peaks that appeared at $2-\theta$, 14.3°, 16.8°, and 22.6° arising from planes ⟨−1 1 0⟩, ⟨1 1 0⟩, and ⟨2 0 0⟩, respectively (Ul-Islam, Khan, & Park, 2012b). An amorphous halo was observed near $2-\theta$ 19°. This was because BC polymer cannot be 100% crystalline and the intensity of this amorphous peak varies from sample to sample. As shown in Table 1, no significant variations were observed in the crystallinity of BC0, BC1, BC2, and BC3. Specifically, the crystallinity of BC0 was 64.34%, while that of BC1, BC2, and BC3

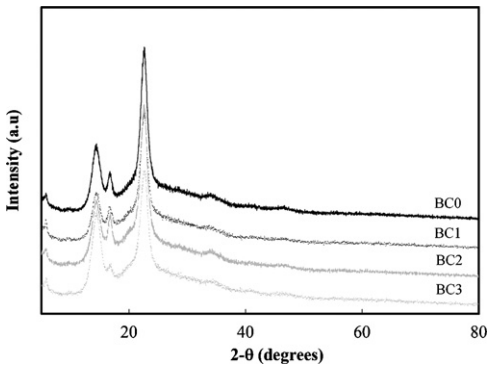

Fig. 6. XRD patterns of BC0, BC1, BC2, and BC3. Samples were produced by cultivation under static conditions at 30 °C for 10 days.

Table 1
d-Spacing, crystallite size, and crystallinity index of BC0, BC1, BC2 and BC3 determined through X-ray diffractograms.

Sample	*d*-Spacing (Å)		Crystallite size (Å)		Cystallinity index (%)
	d_1	d_2	Crystal 1	Crystal 2	
BC0	6.161	5.311	45	86	64.34
BC1	6.122	5.321	49	68	66.41
BC2	6.138	5.288	43	60	69.79
BC3	6.104	5.279	43	63	69.09

was 66.41, 69.79, and 69.09%, respectively. The crystallinity of the samples containing SSGO was higher than that of BC0. The higher crystallinity of the BC1, BC2, and BC3 likely improved their tensile properties. These results showed that the presence of SSGO in the media favored the crystallization process and reduced the relative amorphous region of the BC. The appearance of various peaks at the same position indicates that SSGO present in the media does not disturb the basic crystalline arrangements of BC. However, the crystal size was disturbed to some extent (Table 1). Specifically, it decreased from 86 Å for BC0 to 68, 60, and 63 Å for BC1, BC2, and BC3, respectively. The presence of SSGO might have affected the crystal growth in the ⟨1 1 0⟩ plane.

Another important feature determined from the XRD analyses is the relative amount of two cellulose allomorphs, triclinic (Iα) and monoclinic (Iβ) cellulose. The effect of SSGO on their relative distribution can also be verified from XRD analysis. The ratio of Iα and Iβ depends on the cellulose source. Specifically, peaks obtained from XRD are actually the combination of Iα and Iβ (Yan, Chen, Wang, Wang, & Jiang, 2008). According to studies conducted by Wada, Okano, and Sugiyama (2001), the *d*-spacing values (d_1) of bacterial cellulose should be higher than 6.10 Å and range up to 6.17 Å, while the d_2 values should be below 5.334 Å. The present d spacing values for both d_1 and d_2 (Table 1) showed that all BC samples contained more Iβ cellulose than Iα. The slight decrease in d_1 values for BC1, BC2, and BC3 indicates a relative decrease in the Iβ component in response to the presence of SSGO in the media. Although the decrease in d_1 values was in the limits of the range (above 6.10 Å), showing the dominance of Iβ, the relative amounts of the two allomorphs were altered to some extent with the addition of SSGO to the culture media.

3.4. FT-IR characterization

FT-IR spectroscopy is an important tool for determination of the functional groups and nature of chemical bonds in a molecule (Ul-Islam, Shah, Ha, & Park, 2011). FT-IR analyses of BC0, BC1, BC2 and BC3 were carried out to verify their structure and identify any structural variations caused by the presence of various amounts of SSGO in the media. The combined IR spectra for BC0, BC1, BC2 and BC3 are shown in Fig. 7.

The FT-IR spectra of all samples contained similar peaks, confirming the basic structure of BC. A broad hydrogen bonding peak centered at about ~3350 cm^{-1} was present in all samples. In addition, a sharp band at 2904 cm^{-1} accompanied by another weak band at 2850 cm^{-1} confirmed the C—H stretching vibration (Halib, Iqbal, Amin, & Ahmad, 2012; Ul-Islam et al., 2012b). The presence of the CH group was further supported by the appearance of several peaks corresponding to C—H bending vibrations at 1450–1200 cm^{-1} (Halib et al., 2012; Ul-Islam et al., 2011). The fingerprint region also contained various peaks confirming the BC structure. Specifically, an important peak appeared at around 1050 cm^{-1} in all samples due to the C—O—C stretching vibration (Shah et al., 2010). However, differences in the characteristic bands of BC0 and BC produced in the presence of SSGO media were

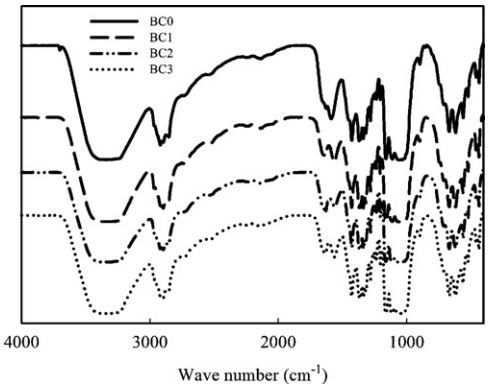

Fig. 7. FT-IR spectral analysis of BC0, BC1, BC2, and BC3. Samples were produced by cultivation under static conditions at 30 °C for 10 days.

observed in the region near 1600 cm^{-1}. Two characteristic peaks appeared for BC1, BC2 and BC3 at 1500–1700 cm^{-1}, while only a single peak was observed for BC0 in the same range. Being hydrophilic in nature, BC adsorb water molecules, resulting in H—O—H bending vibration at about 1600 cm^{-1} (Gunister, Pestreli, Unlu, Atici, & Gungor, 2007). However, the appearance of the second peak seems to be the only visible difference between the FT-IR spectra of BC0 and the rest of the BC samples. The appearance of peaks in this region commonly indicates the presence of a double bond. Although pure BC does not contain any C=O or C=C groups, SSGO contains a definite carboxylic group (Khan & Park, 2008). Accordingly, this peak might appear due to entrapment of certain molecules of SSGO in the BC.

3.5. Mechanical properties

The excellent mechanical properties of BC make it a superior material for use in tissue engineering and wound dressing (Li, Kim, Lee, Kee, & Oh, 2011). Variations in the structure of BC will have a definite effect on its mechanical strength. The mechanical properties of BC0, BC1, BC2 and BC3 were investigated and correlated with the structural mutations. Fig. 8a shows the stress-strain curves of BC0, BC1, BC2 and BC3 samples, while tensile strength, strain and Young's modulus of the samples are shown in Fig. 8b.

The results indicated that the maximum tensile strength at the breaking point for all BC samples produced with the addition of SSGO was higher than for the BC0. The tensile strength for BC0 was found to be 16.73 MPa, which increased to 25.05 MPa for BC1. The tensile strengths of BC2 and BC3 were 20.76 and 19.77 MPa, respectively. The increase in the mechanical strength of BC1, BC2 and BC3 in comparison to that of BC0 could be explained based on the results of SEM analysis. The SEM micrographs (Fig. 5) revealed that the fibers of the BC1, BC2 and BC3 are thicker, more compact and relatively well arranged when compared to BC0. These results in the BC produced in the presence of SSGO having better mechanical properties. As mentioned earlier, the glucose chains aggregate together, resulting in thicker and stronger fibrils. The fibril compactness also increased with the production of more BC in the SSGO amended media. These thick and well arranged fibrils resulted in improved mechanical strength for BC1, BC2 and BC3 relative to BC0, with the highest mechanical strength being observed for BC1. These findings are interesting because the fibril size of BC1 was not the thickest and its density was lower than that of BC2 and BC3. However, the fibrils of BC1 were more uniformly arranged than those of BC0, BC2 and BC3. This good arrangement results in a uniform response to the applied force, and could therefore increase the mechanical strength of BC1. The Young's modulus was also higher for BC1, BC2, and BC3 than BC0, with values of 0.32 GPa for BC0 and 0.71, 0.69 and

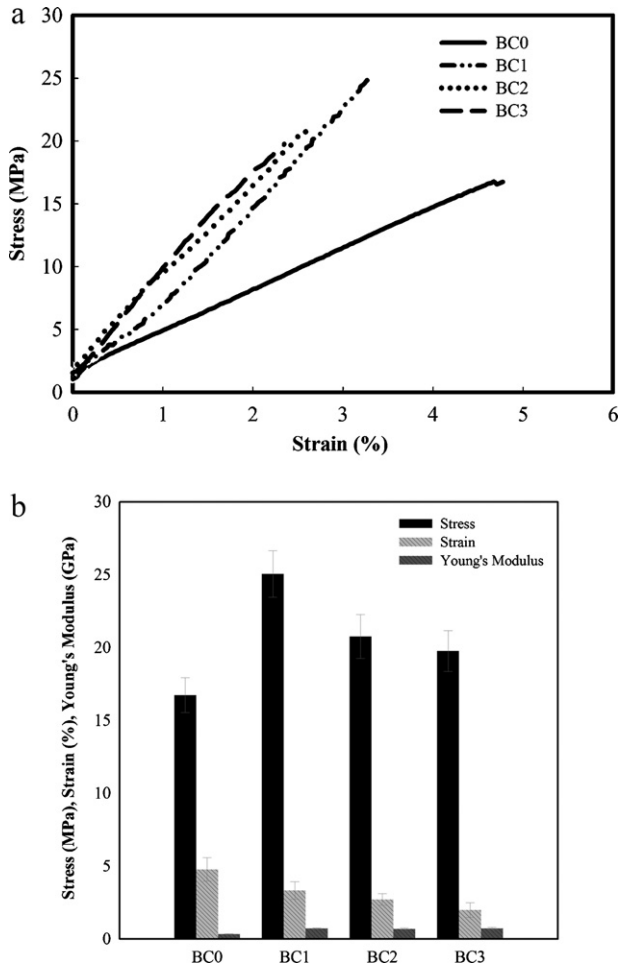

Fig. 8. Mechanical properties of BC0, BC1, BC2, and BC3: (a) Stress strain curves; (b). Tensile stress at breaking point, % strain, and Young's modulus. Samples were prepared by cultivation under static conditions at 30 °C for 10 days.

0.72 GPa for BC2 and BC3, respectively, being observed. The higher Young's modulus values for BC1, BC2 and BC3 indicated improved toughness with the addition of SSGO. However, the elongation at break (strain) was reduced for BC1, BC2 and BC3 when compared to BC0, as indicated by values of 4.77% for BC0 and 3.32, 2.71, and 1.98% for BC1, BC2 and BC3, respectively. The thick and compact fibrils appear to enhance the toughness of the BC and reduce its elasticity. However, the fibrils attain higher tensile strength values with slight modifications to their original structure.

Overall, the results indicated that the mechanical properties of the BC containing added SSGO were higher than those of BC0. This could be an important aspect of the addition of SSGO to the media, which not only improved BC production, but also the mechanical properties of BC.

4. Conclusions

The addition of SSGO to BC synthetic media had a significant effect on its production as well as its mechanical properties. The maximum production was achieved with the addition of 2% SSGO to the synthetic media. The decrease in SSGO quantity indicated that it was used as a carbon source in the production of BC. The fibrils of BC1, BC2 and BC3 were thicker and denser and resulted in higher crystallinity than BC0. The better fibril network arrangement and higher crystallinity subsequently enhanced the mechanical properties of BC1, BC2 and BC3.

Acknowledgements

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