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Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy

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ABSTRACT

The aim of the present study was to investigate the structural modifications and physico-mechanical properties of the BC sheets produced in static cultures using a newly developed fed-batch strategy and a new medium (waste from beer fermentation broth; WBFB). Characteristics of these BC samples were compared with BC sheets produced in batch and fed-batch cultivations using chemically defined medium (CDM). FT-IR and ^{13}C NMR spectra showed almost same results for all the BC samples. XRD analysis revealed that BC produced in fed-batch cultivation using CDM had larger crystallite size than other BC samples. Crystallinity index of BC from fed-batch cultivations was higher than batch cultivation. BC from WBFB had slightly lower degree of polymerization than BC from CDM. Mechanical strength of BC from fed-batch cultivation using CDM was highest. WHC and WRR for BC from WBFB were greater than BC from CDM.

1. Introduction

Cellulose, composed of (1 → 4)-β-D-glucopyranose repeating units, is the most abundant biopolymer on earth. It is the main constituent of plant cell wall. Some kinds of bacteria of the genera Acetobacter, Rhizobium, Agrobacterium, and Sarcina can also produce cellulose called as bacterial cellulose (BC) (Khan, Park, & Kwon, 2007). Recently, BC has received much attention as a new functional material for biomedical and industrial applications because of its superiority to plant cellulose in purity and supermolecular structure. It has unique physical and chemical properties that lack in plant cellulose including high tensile strength, high water holding capacity, high crystallinity, ultra-fine and finely pure fiber network structure, transparency, fiber-binding ability, biocompatibility, biodegradability and moldability (Ishihara, Matsunaga, Hayashi, & Tišler, 2002; Klemm, Schumann, Udhardt, & Marsch, 2001; Shezad, Khan, Khan, & Park, 2009; Vandamme, De Baets, Vanbaelen, Joris, & De Wulf, 1998).

Gluconacetobacter hansenii PJK is known to produce BC under various experimental conditions using a chemically defined medium (CDM) (Jung, Park, & Chang, 2005; Jung, Khan, Park, & Chang, 2007; Park, Hyun, & Jung, 2004; Park, Jung, & Park, 2003; Park, Park, & Jung, 2003; Shah, Ha, & Park, 2010) as well waste from beer fermentation broth (WBFB) (Ha et al., 2008; Park, Hyun, & Ahn, 2006). However, the previous studies were mostly related to the exploration of the fundamental biotechnology and enhancement of productivity in agitated conditions. The agitated culture method is mainly used for industrial production of BC (El-Saied, Basta, & Gobran, 2004) but most of the biomedical (e.g., artificial skin) and cosmoceutical (e.g., face mask) applications require BC to be in a proper shape (e.g., film, membrane or sheet), which can be produced only in static cultivations (Park et al., 2009). The process by which BC is conventionally produced using a static culture is not applicable to large-scale industrial production due to the low productivity. In order to overcome this low productivity problem in static cultures and to enhance it to a level suitable for commercial applications, a simple fed-batch strategy was successfully applied for the production of BC sheets in a fermenter using CDM and WBFB (Shezad et al., 2009). The produced BC sheets appeared to be applicable for biomedical and other applications.

It is known that the composition of the culture medium and fermentation conditions greatly influence the chemical structure, composition and viscosity of the microbial polysaccharides including BC (Duta, França, & Lopes, 2006; Khan & Park, 2008; Nieduszynski & Preston, 1970; Watanabe, Tabuchi, Morinaga, & Yoshinaga, 1998). These modifications (if any) in turn may affect

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the properties of BC. Therefore, it seemed important to investigate the possible modifications in the physicochemical properties of the BC sheets that may have been produced using a new strategy (fed-batch) and using the new medium (WBFB) employed in our previous studies (Shezad et al., 2009). For this purpose, the BC sheets produced in fed-batch cultivation using CDM and the WBFB were subjected to detailed structural analyses using FT-IR, XRD, CP/MAS ^{13}C NMR and gel permeation chromatography while the various physical properties investigated included morphology, mechanical strength, water holding capacity and water release rate. These properties were compared with the physicochemical properties of BC produced in batch cultivation using CDM. This study will be helpful in determining if the BC obtained in the new conditions is appropriate for commercial applications.

2. Experimental

2.1. Production and processing of BC sheets

The BC sheets used in this study were obtained in previous investigations via batch and fed-batch cultivation strategy using CDM and WBFB (Shezad et al., 2009). Briefly, the colonies of *G. hansenii* PJK (KCTC 10505BP) were inoculated into a 50 mL CDM (containing 10 g glucose, 10 g yeast extract, 7 g peptone, 1.5 mL acetic acid, and 0.2 g succinate dissolved in 1 L of distilled water and its pH adjusted to 5) in a 250 mL flask which was shaken at 200 rpm and cultured at 30 °C for 24 h. For batch cultivation, 100 mL pre-culture was inoculated into 2 L CDM containing 1% ethanol in a 3 L jar fermenter. In fed-batch cultivation, 25 mL pre-culture was inoculated into 500 mL of culture medium (CDM or WBFB) in a 3 L jar fermenter. A fresh medium (250 mL) was fed periodically. In either case, the fermentations were carried out at 30 °C with an aeration rate of 1 vvm.

The BC sheets were harvested after 30 days cultivation in each case and washed with distilled water repeatedly in order to remove the residual medium and other impurities. These BC sheets were then repeatedly treated by boiling in 0.3 N NaOH solution for 15–20 min followed by washing with distilled water. Finally, the sheets were stored in containers containing distilled water while for characterization; portions of the BC sheets were freeze-dried.

2.2. FE-SEM analysis

In order to determine the morphology and surface topography of the BC sheets, micrographs of the platinum-coated samples of freeze dried BC were taken with a field-emission scanning electron microscope (S-4300; Hitachi Co., Japan).

2.3. XRD analysis

The XRD technique was employed in order to determine the crystallite size and crystallinity of the BC samples. The freeze-dried BC samples were studied using a Rigaku D/max 2500 X-ray diffractometer with a thin film attachment and operated at room temperature. The Cu Kα X-ray source was set to 40 kV and 100 mA. The samples were scanned from 10° to 80° with a scan speed of 4°/min. The data were obtained with the help of a MDI/JADE6 software package attached to the Rigaku XRD instrument. The Scherrer's formula:

$$\text{Crystallite Size} = \frac{k\lambda}{W}\cos\theta$$

(with a shape factor $k = 0.89$) was employed to determine the crystallite sizes of samples with full width at half maximum (fwhms, W) and peak centers obtained by fitting the (1 0 1) peak to the Lorentzian function; here λ is the wavelength of X-ray radiation (1.54 Å) (Song et al., 2009).

The crystallite index (CrI^{XRD}) was calculated with the help of the following formula:

$$\text{CrI}^{\text{XRD}} = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$

where I_{200} is the maximum intensity of the (2 0 0) lattice diffraction and I_{am} is the intensity diffraction at 2θ (Focher et al., 2001).

2.4. FT-IR analysis

The surface properties of the BC samples were examined by a Perkin-Elmer S2000 Fourier transform infrared spectrometer (FT-IR, Nicolet Magna IR 560) in an attenuated total reflectance (ATR) mode. BC samples were milled with KBr to form a very fine powder which were then compressed into a thin pellet and analyzed. All FT-IR spectra were recorded in the transmittance mode in the range of 4000–400 cm^{-1} with a resolution of 0.25 cm^{-1}.

2.5. Cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR spectroscopy

The CP/MAS ^{13}C NMR spectra were recorded (at 294 ± 1 K) on the Bruker Avance II^{+}-400 instrument operating at 9.4 T. A double air-bearing probe and a zirconium oxide rotor were used. The MAS rate was in the range of 6–10 kHz. Acquisition was performed with a standard CP pulse sequence using a 4.2 μs proton 90 pulse, a 2000 μs contact pulse and 3 s delays between repetitions. Adamantine was used as an external standard for the chemical shift scale relative to tetramethylsilane.

2.6. Gel permeation chromatography (GPC)

Weight average molecular weight (Mw), polydispersity index (Mw/Mn) and degree of polymerization (DP) for the nitrated BC samples were determined by a high-performance gel permeation chromatography system (GPCV 2000, Water Alliance, USA) according to the technique described elsewhere (Kuga et al., 1989). Each cellulose sample was nitrated in a solution of fuming nitric acid/diphosphorous pentaoxide according to the method of Alexander and Michell (1949). Briefly, the nitrating mixture was prepared by dissolving 120 g of phosphorous pentaoxide in 300 g of nitric acid (90%, Sigma–Aldrich). Then 1 g BC was introduced into nitrating mixture (40 g), and reacted at 20 °C for 20 min with the sample being swirled at about 5 min intervals. The resulting BC product was thoroughly washed with cold (10 °C) distilled water and neutralized with 5% sodium carbonate solution followed by three washes with distilled water. The BC product was then boiled in distilled water for 20 min and soaked in methyl alcohol (50 mL) for 10 min. The final product was obtained by drying the BC samples in a mechanical convection oven at 50°C for about 1 h. The molecular weight of the samples was checked with GPC using tetrahydrofuran as eluent.

2.7. Mechanical strength

The tensile properties of the BC membranes were measured using an Instron Universal Testing Machine (Model 4465, USA) according to the procedure of American Society for Testing and Materials (ASTM D 882). Two metal clamps were placed at either end of a 100 mm × 10 mm rectangular strip of dried BC sample in each case. The clamps were then mounted on an Instron 4465 that measured the maximum tensile strength before fracture. Thickness of each sample was measured by using a Peacock Model G (made in Japan).

2.8. Measurement of water holding capacity (WHC) and water release rate (WRR)

The water holding capacity of the samples was measured using the shake method (Schrecker & Gostomski, 2005). Three BC samples for each batch (with the dimension 7.5 cm × 5 cm × 0.8 cm were removed from the storage container using tweezers. The samples were shaken twice quickly and then weighed. These samples were dried at room temperature for 48 h and their weights were measured at different time intervals. Then, BC samples were dried for 12 h at 60 °C in order to completely remove water from them. Finally, they were transferred quickly to the balance for weighing. The water holding capacity was calculated using the following formula:

$$\text{Water Holding Capacity} = \frac{\text{Mass of water removed during drying (g)}}{\text{Dry weight of cellulose (g)}}$$

For the determination of the water release rate, the wet weight of BC samples was measured followed by continuously weighing the samples stored at ambient conditions at different time intervals. Finally the dry weight of the respective BC samples was taken which was subtracted from all the readings. Similarly the loss of water at different time intervals was plotted against time.

3. Results and discussion

3.1. Morphology of BC sheets prepared under various conditions

BC is accumulated at the surface of the culture medium as a gelatinous membrane in a static culture (Hestrin & Schramm, 1954). Nascent chains of BC aggregate to form subfibrils which are then crystallized into microfibrils, these into bundles, and the latter into ribbons (Park et al., 2009).

Fig. 1 shows the scanning electron micrographs of the BC sheets produced in different cultivation modes and media. These micrographs clearly show the various morphological features of these sheets. All samples showed a reticulated structure consisting of ultra fine cellulose fibrils. The gross morphological structure seemed to be similar for all specimens. However, detailed examination of the micrographs revealed profound morphological differences among the nanofibrils and the reticulated structures of BC produced under different culture conditions. Depending on their origin, these BC nanofibrils have transverse dimensions that range from 10 to 180 nm. The fibrils of BC produced in fed-batch cultivation using CDM are highly extended and almost uniform in size. In contrast, the fibrils of BC sheets produced in batch cultivation appeared to be smaller in size. The fibrils of BC produced in fed-batch cultivation using WBFB are more crowded and thinner than BC fibrils produced using CDM. The exact width of individual microfibrils in each case, however, is difficult to estimate. The morphological changes in BC sheets affect the microstructures and various properties including the degree of polymerization, crystallinity, content of cellulose I_α and water holding capacity (Park et al., 2009; Watanabe et al., 1998).

3.2. XRD analysis

The morphological changes in the ribbons of BC are related to the changes in microstructures such as crystallinity, I_α fraction and hydrogen holding between the molecules (Sun et al., 2007; Watanabe et al., 1998). In order to compare the microstructural changes in the BC sheets produced under different cultivation modes and conditions, X-ray diffraction was used. The diffraction

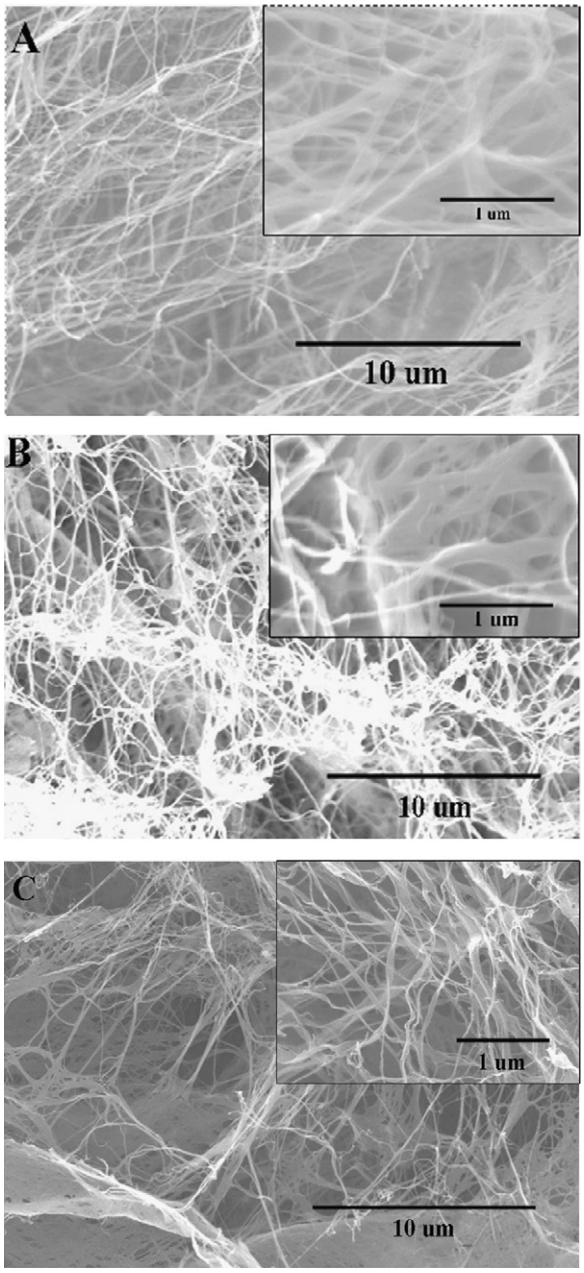

Fig. 1. FE-SEM micrographs of bacterial cellulose samples produced in batch cultivation using chemically defined medium (A), fed-batch cultivation using chemically defined medium (B), and fed-batch cultivation using waste from beer fermentation broth (C) in static conditions in a Jar fermenter.

diagram indicated that BC synthesized in different culture conditions has both I_α and I_β crystal cellulose. Fig. 2 revealed three characteristic diffraction peaks in the region of 10–35° under different conditions while the crystalline parameters of (0 0 2) crystal plate of BC synthesized in different culture modes are listed in Table 1.

Fig. 2 represents X-ray diffraction patterns of BC samples produced using CDM in batch and fed-batch cultivation modes and WBFB in fed-batch cultivation. These patterns are apparently similar to each other which are based on the BC polymorphs examined in this study. The fiber diagram of all the cellulose samples is well resolved pattern of the I_α rich type of cellulose (55.58, 58.94 and 58.73% of I_α phase respectively), which identifies the cellulose production by bacteria. However, there are some slight differences in their structural features. Fig. 2B and C approximately shows simi-

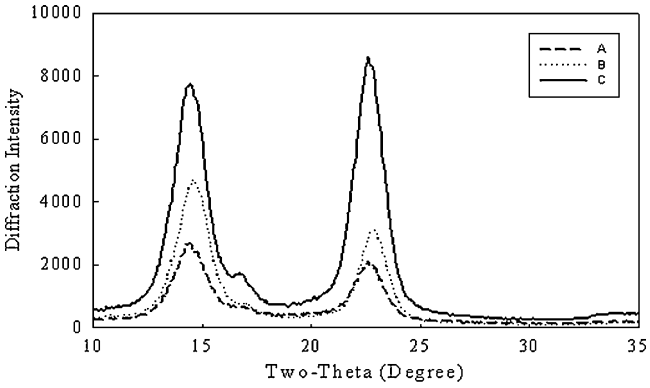

Fig. 2. X-ray diffraction patterns of bacterial cellulose samples produced in batch cultivation using chemically defined medium (A), fed-batch cultivation using chemically defined medium (B), and fed-batch cultivation using waste from beer fermentation broth (C) in static conditions in a Jar fermenter.

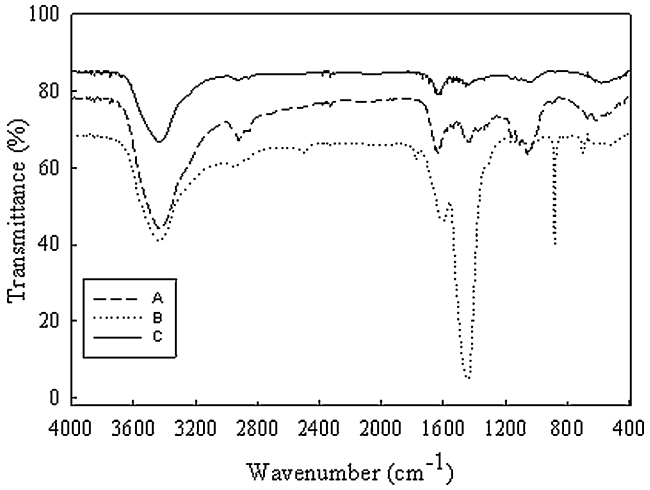

Fig. 3. FT-IR spectrum of bacterial cellulose samples produced using chemically defined medium in batch cultivation (A), chemically defined medium in fed-batch cultivation (B), and waste from beer fermentation broth in fed-batch cultivation (C) in static conditions in a Jar fermenter.

lar cellulose I_α and cellulose I_β mass fraction while Fig. 2A shows slightly lower mass fraction of cellulose I_α. The former two have been produced in fed-batch cultivation while the later in batch cultivation mode. This shows that cultivation mode has an effect on cellulose I_α and cellulose I_β mass fraction, while change in culture medium has no significant effect. Moreover, the d-spacing is almost the same in all the cases.

BC produced in fed-batch cultivation using CDM has larger crystallite size of a crystallographic plane (1 1 0) than BC produced in batch cultivation using CDM or fed-batch cultivation using WBFB (Table 1). The latter sample has the smallest crystal size. Similarly, the crystallinity index of BC samples produced in fed-batch cultivation using WBFB is highest. WBFB is a complex natural medium consisting of several ingredients. It may also contain some constituent(s) which can serve as a dispersant to lessen the aggregation of particles. The cultivation conditions may also affect the aggregation of particles.

From these results it can be concluded that the cultivation mode has a greater effect on cellulose I_α and cellulose I_β mass fraction, crystallite size and crystallinity index. However, the culture medium has a significant effect on crystallite size and crystallinity index.

3.3. FT-IR analysis

Fourier transform infrared (FT-IR) spectra of BC samples were taken in order to detect any peak shift that could be attributed to BC produced in different media and cultivation modes. Fig. 3 shows the FT-IR spectra of BC produced in different media and cultivation modes. Table 2 indicates the characteristic bands of BC synthesized in different modes and media appeared at ~3430–3435 cm^{-1} for hydroxyl groups stretching vibration, at ~2927–2949 cm^{-1} for C–H stretching vibration, at ~1433–1456 cm^{-1} for C–H bending vibration and at ~1045–1067 cm^{-1} for C–O–C and C–O–H stretching vibration of sugar ring (Socrates, 2001; Sun et al., 2007). This carbonyl amide group may come from proteins and bacterial cells which are not completely wiped off after the NaOH treatment of the BC membrane (Sun et al., 2007). The carbonyl amide peak was shorter in the spectra of BC produced in fed-batch cultivation using WBFB or in the batch cultivation using CDM than the in fed-batch cultivation using CDM. This means that the former two samples are

Table 1
d-Spacing, crystallite sizes and cellulose I_α and I_β content (%) of different bacterial cellulose samples determined from X-ray diffractograms.

BC sample	*d*-Spacing (Å)		Crystallite sizes (nm)		Cellulose type (%)		Crystallinity index (%)
	Dl	D2	Crystal 1	Crystal 2	I_α	I_β	
Batch cultivation (CDM)[b]	6.12[a]	3.93[a]	10.37	6.86	55.58	44.41	78.0
Fed-batch cultivation (C DM)[b]	6.18[a]	3.91[a]	10.95	7.22	58.94	41.05	75.5
Fed-batch cultivation (WBFB)[b]	6.13[a]	3.94[a]	9.07	5.74	58.73	41.26	80.8

[a] Where *d* is the perpendicular distance sepamting each lattice plane in a stack.
[b] *Abbreviations*: CDM, chemically defined medium; WBFB, waste from beer fermentation broth.

Table 2
Assignment of FT-IR spectra of bacterial cellulose samples produced at various culture conditions.

Band assignments	Wave number (cm^{-1})		
	Batch cultivation (CDM)[a]	Fed-batch cultivation (CDM)[a]	Fed-batch cultivation (WBFB)[a]
O–H stretching	3430	3435	3435
C–H stretching	2922	2964	2923
C–H bending	1456	1443	1456
C–O–H bending, C–OH stretching	1060	1070	1056
–CO–NH	1641	1600	1632
O–D stretching	Negligible	2489	Negligible
–CO–Br	Negligible	1772	Negligible
N–H out of plan bending	Negligible	885	Negligible

[a] *Abbreviations*: CDM, chemically defined medium; WBFB, waste from beer fermentation broth.

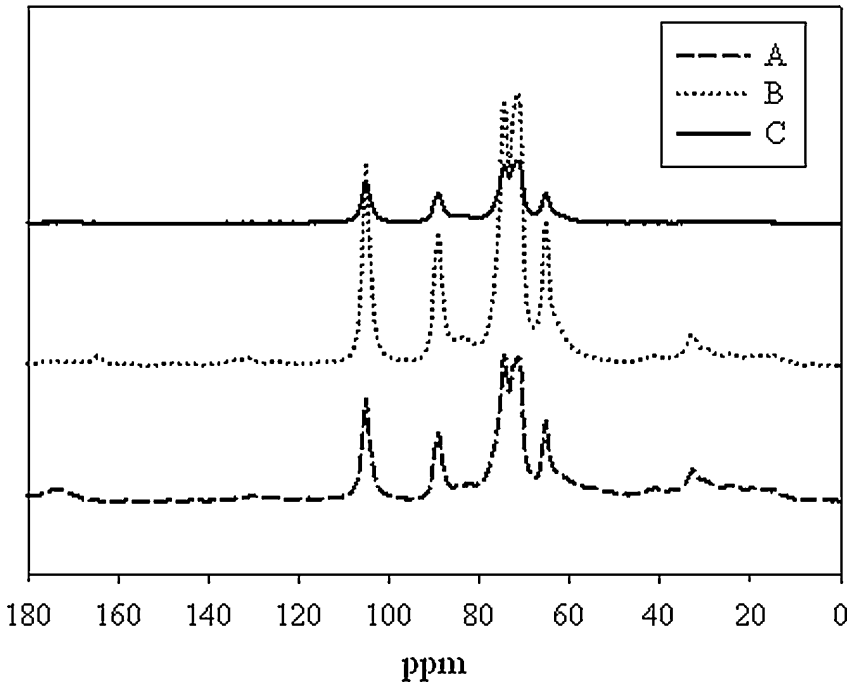

Fig. 4. The CP/MAS ^{13}C NMR spectra of bacterial cellulose samples produced in batch cultivation using chemically defined medium (A), fed-batch cultivation using chemically defined medium (B), and fed-batch cultivation using waste from beer fermentation broth (C) in static conditions in a Jar fermenter.

more pure than the later one. The band position in the FT-IR spectra for all the three BC samples were in the same range and did not show any significant difference.

Three additional dominant peaks are found in the spectra of BC produced in fed-batch cultivation using CDM as shown in Table 2, at ~2489 cm^{-1} for O–D stretching (Socrates, 2001), at 885 cm^{-1} for N–H out of plan bending (Pavia et al., 2001) and at ~1772 cm^{-1} for acid halide (–CO–Br) (Socrates, 2001).

3.4. CP/MAS ^{13}C NMR spectroscopy

CP/MAS is a useful technique for monitoring the morphological changes taking place in cellulose during processing (Bhattacharya, Louis, & William, 2008). Therefore, this technique was applied in the present study in order to monitor the changes in cellulose caused by the changes in the fermentation conditions. ^{13}C NMR spectra of BC produced in batch and fed-batch cultivation using CDM and fed-batch cultivation using WBFB are presented in Fig. 4. The peaks for the six carbon atoms of the cellulose molecule were dominant in the spectra in all cases. The chemical shift values ranged from 105 to 65 ppm. The anomeric carbon (C_1) appeared at around 105 ppm followed by the signal from the C_4 atom at around 89 ppm. Peaks arising due to C_2, C_3 and C_5 atoms make their appearance between 70 and 75 ppm and finally the C_6 peak has a chemical shift value at around 65 ppm. These values are in agreement with those reported in the literature for cellulose (Bhattacharya et al., 2008).

In previous studies on solid-state NMR spectra of cellulose two peaks appeared in the chemical shift range 80–92 ppm which were assigned to the C_4 carbon atom (Atalla, Gast, Sindorf, Bartuska, & Maciel, 1980; Earl & VanderHart, 1981). The relatively sharp peak was due to crystalline regions while broader peak was assigned to the crystallite surfaces and the amorphous/disordered domains (Atalla et al., 1980; Earl & VanderHart, 1981). In contrast, a single and sharp peak at 89 ppm, assigned to C_4, was obtained in the current study which showed the crystalline nature of the obtained BC. The correlation of the cellulose NMR spectra to its structure and morphology was also revealed by the assignments reported previously (Horii, Hirai, & Kitamaru, 1984; Newman, 1998; Newman & Hemmingson, 1995; Wickholm, Larsson, & Iversen, 1998). A slight shoulder on the C_6 peak, between 63 and 65 ppm, was attributed to the amorphous and disordered component in cellulose (Horii et al., 1984; Newman, 1998; Newman & Hemmingson, 1995; Wickholm et al., 1998). Such a shoulder is absent in the spectra obtained in the present studies.

However, two uncommon peaks are found in ^{13}C NMR spectra of BC produced in batch and fed-batch cultivation using CDM as shown in Table 3. The uncommon peaks at 173.81, 164.48 ppm represent the presence of carbonyl amide group (Pavia et al., 2001; Sun et al., 2007). These peaks are negligible in the spectra of BC produced in fed-batch cultivation using WBFB. The higher chemical shift value for BC obtained in batch compared to fed-batch cultivation using CDM represents the presence of secondary or tertiary amine attached to the carbonylic carbon. The lower chemical shift value for BC obtained in fed-batch cultivation using CDM represents the possible presence of primary amine attached to the carbonylic carbon. The electron withdrawing effect of tertiary/secondary amine group is higher compared to primary amine (Pavia et al., 2001) hence caused the high chemical shift in case of BC produced in batch cultivation. This can be coincident with a relatively evident band at 885 cm^{-1} in FT-IR spectra for N–H out of plan bending in case of BC produced in fed-batch cultivation using CDM. Similarly, the peaks at 32.87 ppm are also dominant for BC produced using CDM which indicate the presence of saturated carbon attached to the carbonyl amide group (Pavia et al., 2001). The overall studies of NMR and FT-IR spectra indicate that the representative peaks for carbonyl groups attached with amines are dominant for BC produced using CDM compared to WBFB. The slight reduction in wave number for O–H stretching and an increase in chemical shift for BC produced using CDM compared to WBFB indicate the presence of traces of bacterial bodies in the BC even after treatment with NaOH solution.

Hence, from this expanded NMR and FT-IR studies given in Tables 2 and 3, both the spectra argue for the presence of some proteinacious bodies which gave rise to additional peaks. The spectra clarify that the peaks for carbonyl amide are relatively dominant for BC produced using CDM compared to WBFB. Hence, this

Table 3
^{13}C Chemical shifts of bacterial cellulose samples produced at various culture conditions.

Culture type	^{13}C chemical shifts (ppm)					
	C_1	C_2, C_3, C_5	C_4	C_6	R–CO–NH_2	Saturated card on
Batch cultivation (CDM)[a]	105.11	70–75	89.00	65.29	173.81	32.81
Fed-batch cultivation (CDM)[a]	105.11	70–75	89.03	65.29	164.48	32.81
Fed-batch cultivation (WBFB)[a]	105.13	70–75	89.03	65.27	Negligible	Negligible

[a] *Abbreviations*: CDM, chemically defined medium; WBFB, waste from beer fermentation broth.

support that the thick and long fibrils of BC produced using CDM hold the bacterial cells more firmly and acted as a barrier during washing which resulted in the remain of some bacterial cell debris.

3.5. Degree of polymerization

Molecular weight is an important property of polymeric materials (Choi, Song, Kim, Chang, & Kim, 2009). Many of the unique physical properties of BC are attributed to the high molecular weight, molecular homogeneity and chemical purity. In the current study the Mw characterization of BC samples were carried out using the GPC method. This method furnishes comprehensive information regarding all important molecular parameters of the polymer, that is, number and weigh-average molecular weights (Mn and Mw), polydispersity (Mw/Mn) and degree of polymerization (Mn/monomer molecular weight).

The retention times of BC samples produced in batch and fed-batch cultivation using CDM, and fed-batch cultivation using WBFB were 22.450, 22.540, and 22.542 min, respectively. Fig. 5 shows the degree of polymerization patterns of BC samples produced in different culture conditions. It is obvious from these results that BC samples obtained from fed-batch cultivation using WBFB has slight lower DP than BC produced in batch and fed-batch cultivation using CDM. As shown in Table 4, the highest DP value (8011) was obtained for the BC produced in batch culture using CDM. The lowest DP value for BC produced in fed-batch cultivation using WBFB may be due to the fact that WBFB is a complex natural medium composing of several ingredients. It may also contain some constituent(s) which can serve as dispersant to lessen the aggregation of particles or constituent(s) which may interferes with the hydrogen bonding between microfibrils resulting in a reduced length of the microfibrils, and thus the Mw of BC (Choi et al., 2009). The cultivation conditions may also effect the aggregation of particles.

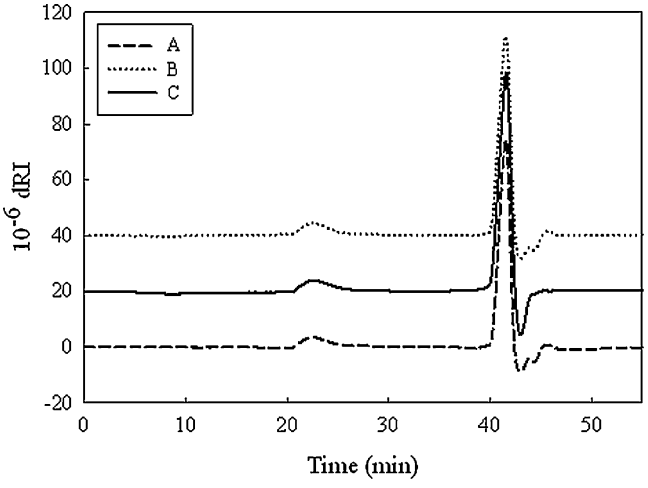

Fig. 5. GPC analysis of bacterial cellulose samples produced in batch cultivation using chemically defined medium (A), fed-batch using chemically defined medium (B), and fed-batch using waste from beer fermentation broth (C) in static conditions in a Jar fermenter.

Generally, the higher the molecular weight of a polymer, the higher is its mechanical strength. The mechanical strength of the BC with high DP will be greater than that of BC with low DP. The polydispersity index (PDI), an index of polymeric uniformity, of BC produced in all culture condition is similar (Table 4).

3.6. Mechanical strength

Tensile testing was used to evaluate the inherent mechanical properties of the produced BC sheets and to determine the effect of various culture conditions on the mechanical properties. Table 4 shows the maximum tensile stress of BC sample obtained by batch cultivation using CDM and fed-batch cultivation using WBFB and CDM. The results displayed that the mechanical strength of BC sample obtained by fed-batch cultivation using CDM is highest compared to fed-batch cultivation using WBFB and batch cultivation using CDM. The GPC results (Fig. 5 and Table 4) showed that the degree of polymerization for batch cultivation using CDM is greater than fed-batch cultivation using CDM and WBFB. As discussed earlier, DP has a greater effect on the mechanical strength. The mechanical strength of BC with higher DP will be greater than BC with lower DP (Cheng, Catchmark, & Demirci, 2009). However, the results obtained for mechanical strength of BC samples produced under various culture conditions do not obey this theory. The discrepancy can be explained from the FE-SEM images. The fibrils of BC sample produced using batch cultivation using CDM are less uniform and smaller in size as compared to the BC samples produce in fed-batch cultivation and thus possess the least mechanical strength. Similarly, the fibrils of BC produced in fed-batch cultivation using WBFB are also less uniform in size but they are more crowded and thus possess the higher mechanical strength. The mechanical strength of BC sheets also depends on the orientation of the micro-fibrils in ribbons (Keshk, 2006; Yano, Maeda, & Nakajima, 2008). BC sheets produced in fed-batch cultivation using CDM have considerable uniplanar orientation of micro-fibrils compared to other BC samples. This may be another basic reason for the high mechanical strength of BC sheets produced in fed-batch cultivation using CDM.

3.7. Water holding capacity (WHC) and water release rate (WRR)

Maintenance of proper wound moisture is important in modern therapy because the penetration of active substances into the wound is facilitated by a moist environment. This also enables an easy and painless dressing change without damage to the newly formed skin (Newman, 1998). In order to estimate the usefulness of the BC sheets produced under various cultivation conditions in the treatment of wounds requiring wet conditions and frequent dressing change, their water holding capacity (WHC) and water release rate (WRR) was determined.

The WHC for the BC sample produced in fed-batch cultivation using CDM was found to be 273 times its dry weight while the WHC for batch cultivation was 296 times its dry weight. This means that WHC for BC produced in batch cultivation is higher than the BC produced in fed-batch using chemically defined medium. However, the reverse was true for WRR i.e., release of water from fed-batch BC

Table 4
Molecular weight and its distribution for bacterial cellulose produced at various culture conditions.

BC sample	Mn[a]	Mw[a]	Mp[a]	PDI[a]	DP[a]	Max tensile stress (MPa)
Batch cultivation (CDM)[a]	1,297,924	1,968,687	3,020,792	1.516797	8011	76.7
Fed-batch cultivation (CDM)[a]	1,286,410	1,945,804	2,766,903	1.512584	7940	19.8
Fed-batch cultivation (WBFB)[a]	1,250,774	1,914,041	2,753,342	1.530285	7720	4.0

[a] *Abbreviations*: Mn, number average molecular weight; Mw, weight average molecular weight; Mp, molecular weight peak at top; PDI, polydispersity index (Mw/Mn); DP, degree of polymerization; CDM, chemically defined medium; WBFB, waste from beer fermentation broth.

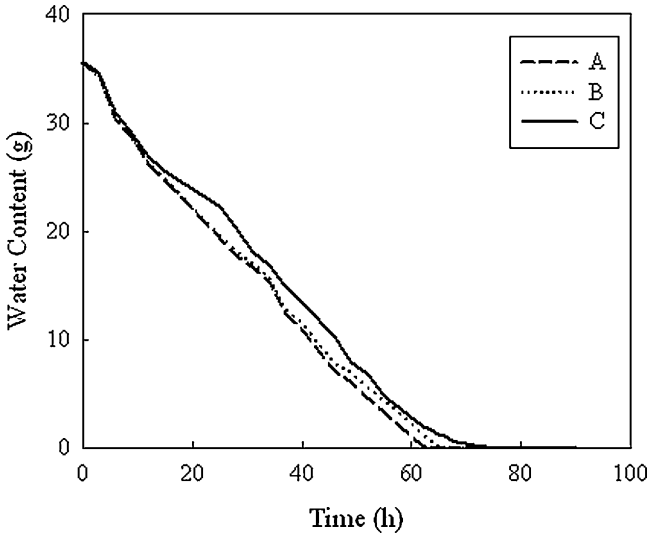

Fig. 6. Water release rate for bacterial cellulose samples produced in batch using chemically defined medium (A), fed-batch cultivation using chemically defined medium (B), and fed-batch cultivation using waste from beer culture broth (C) in static conditions in a Jar fermenter.

took more time as compared to batch cultivated BC sample (Fig. 6). After 64 h almost all of the water was released from the batch sample, while in case of the fed-batch sample water was completely released after 75 h. As revealed from SEM morphology that fed-batch BC fibrils are thicker in size as compared to batch BC fibrils. Therefore, the water molecules are sandwiched between the large and thick fibrils of fed-batch BC, and these fibrils act as a shield for water molecules. Hence, these fibrils prevent the water from moving out of the BC membrane at high speeds. The fibrils of batch cultivation BC are smaller, and having a large surface area. Thus holding more water as compared to fed-batch BC sample. The WHC of fed-batch BC sample using WBFB was found to be 302 times its dry weight and is larger when compared to the BC samples from the chemically defined medium (both batch and fed-batch BC). Ougiya, Watanabe, Matsumura, and Yoshinaga (1998) reported that BC samples with thinner and longer fibrils have high WHC capacity. The high WHC of BC produced in fed-batch cultivation using WBFB as culture medium may be due to thinner and more crowded fibrils.

The amount of the bound water in bacterial cellulose was reported to be negligible (Wickholm et al., 1998) and this water is trapped physically at the surface and on the inside of the particles composed of the reticulated fibrils (Watanabe et al., 1998). The slow release of water from BC is important in biomedical applications i.e., wound dressing (Ciechańska, 2004; Okiyama, Motoki, & Yamanaka, 1992). In this context, the BC produced using WBFB seems to be superior to that produced using chemically defined medium.

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