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Surface Properties of Bacterial Nanocellulose Using Spectroscopic Methods and X-Ray Diffraction

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Abstract: In this study, surface properties of bacterial nanocellulose, harvested from different culture media, with and without ethanol, using spectroscopic methods and X-ray diffraction were investigated. The addition of ethanol resulted in BNCs with high O/C ratios. XRD and FT-IR measurements showed a higher crystallinity for the BNC grown in H medium, while BNC grown in Z medium containing ethanol contained more surface carboxylate groups. This study shows that surface properties of BNC can be altered by the culture medium and ethanol.

Keywords: Bacterial Nano-Cellulose, EDS, FT-IR Spectroscopy, X-Ray Diffraction

1. Introduction

Cellulose, the most abundant bio-macromolecule, is predominantly produced by vascular plants [1]. It is composed of glucose monomers connected by β (1–4) glycosidic linkages to form long chains, with a degree of polymerization >5000. Because of the increased demand for natural cellulose and increased consumption of wood as a raw material of cellulose, deforestation is occurring worldwide and creating global environmental issues [2]. Therefore, an alternative source for cellulose production is necessary.

Bacterial nano-cellulose (BNC) is an exopolysaccharide produced from various species of bacteria, such as those of the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* [3].

G. xylinus is an efficient bacterial species for producing BNC at large scale. BNC possesses special and unique properties. It is chemically pure (free of other structural components, such as lignin and hemicelluloses), has a highly crystalline nano-structure, and a degree of polymerization that distinguishes it from other forms of cellulose [4]. Therefore, BC represents a promising alternative to plant-derived cellulose for specific applications in bio-medicine, cosmetics, high-end acoustic diaphragms, papermaking, food industry and other applications.

In this study, surface properties of bacterial nanocellulose, harvested from different culture media, using spectroscopic methods and X-ray diffraction were investigated.

2. Experimental

G. xylinus was obtained from the Persian Type Culture Collection (PTCC), strain number 1734. The strain was cultured on glucose yeast extract (GYE) agar containing D-glucose (100 g), yeast extract (10 g), peptone (5 g), $CaCO_3$ (20g), agar (25g) per L [5].

Pre-culture medium (50 ml) composed of H medium [6] was placed in a 250 ml Erlenmeyer flask, and autoclaved at 121°C for 15 min before inoculation. Then, the flask was cooled at room temperature, inoculated with the stock culture (a loop from a slant), and incubated in a shaker (150 rpm and 28°C) for three days. Figure 1 shows the bacteria growth within 3 days of incubation. As can be observed, rod-shaped bacteria were grown in number and length dramatically.

After, Hestrin-Schramm (H), Yamanaka (Y) and Zhou (Z) media containing glucose as carbon source, with (+) and without (-) ethanol, were cultured in 250 ml flasks

containing 50 ml of medium [7, 8].

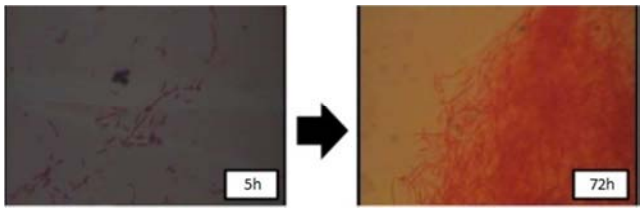

Figure 1. *Bacteria growth after 3 days of incubation.*

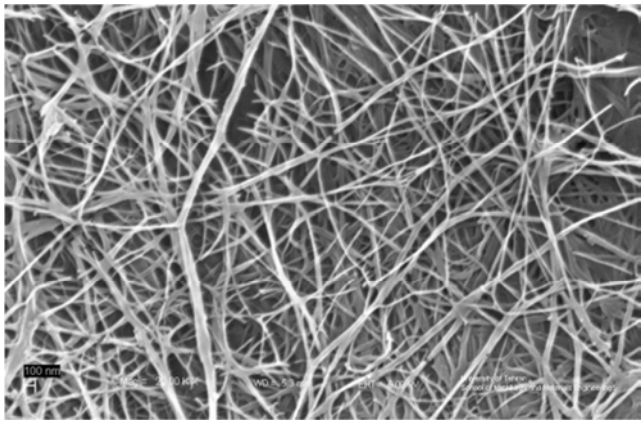

Figure 2. *FE-SEM micrograph of produced BNC.*

After the incubation period, the harvested BNC was initially washed with 1% NaOH at 80°C for 1 h, and then with distilled water repeatedly until a neutral pH was reached. Finally, bacterial nanocellulose samples were oven dried at 45°C for three days and then weighed. Figure 2 represents the FE-SEM micrograph of produced BNC. The analysis performed did not show the existence of significant differences among the dimensions of nanofibers obtained from the different media, with nanofibers widths in the range of 29–77 nm, and thickness values in the 15–31 nm intervals.

Fourier transform infrared spectroscopy (FTIR) of dried BNC was performed on a Bruker Equinox 55 analyzer, equipped with a DTGS detector and a golden gate micro ATR. The spectra were collected at wave numbers ranging between 4000-600 cm^{-1} with an average of 16 scans. In addition, I_α and I_β contents were calculated using the peak heights at 750 and 710 cm^{-1} [9].

The elemental composition of the samples was determined by energy-dispersive X-ray spectroscopy (EDS). Quantitative analyses were done, in triplicate, for both weight (wt%) and atomic (at%) percentages for carbon (C), oxygen (O), nitrogen (N), and O/C ratio.

X-ray diffraction patterns of BNC (1 cm^2) were collected on an X'Pert pro MPD (multi-purpose diffractometer, Model PW3040/60) with CuK_α radiation generation at a temperature of 25°C, resolution of 0.001°, voltage of 40 kV and filament emission of 40 mA. Diffraction intensities were measured between 2θ of 5-50°. Crystallinity (Cr%) was calculated by the following equation:

$$Cr(\%) = \left(S_c / S_t\right) \times 100 \quad (1)$$

where: S_c and S_t are the areas of the crystalline and total domains, respectively. Crystallite size (CrS) was estimated using the Scherrer equation:

$$CrS = K\lambda / \beta \cos\theta \quad (2)$$

where K is the shape factor (0.9), λ is the x-ray wavelength (1.54Å), β is the line broadening at half the maximum intensity (FWHM) in radians and θ is Bragg's angle.

3. Results and Discussion

3.1. Fourier Transform Infrared (FT-IR) Spectroscopy

The structure of BNC was determined by FTIR spectroscopy (Figure 3). The main band assignments are given in Table 1. The bands observed between 3488 cm^{-1} and 3447 cm^{-1} are characteristic of intramolecular hydrogen bonded O-H stretching for cellulose [10]. The band intensity at 3345 cm^{-1} of BNC harvested from medium H+ was more intense than those of BNC from the other media types.

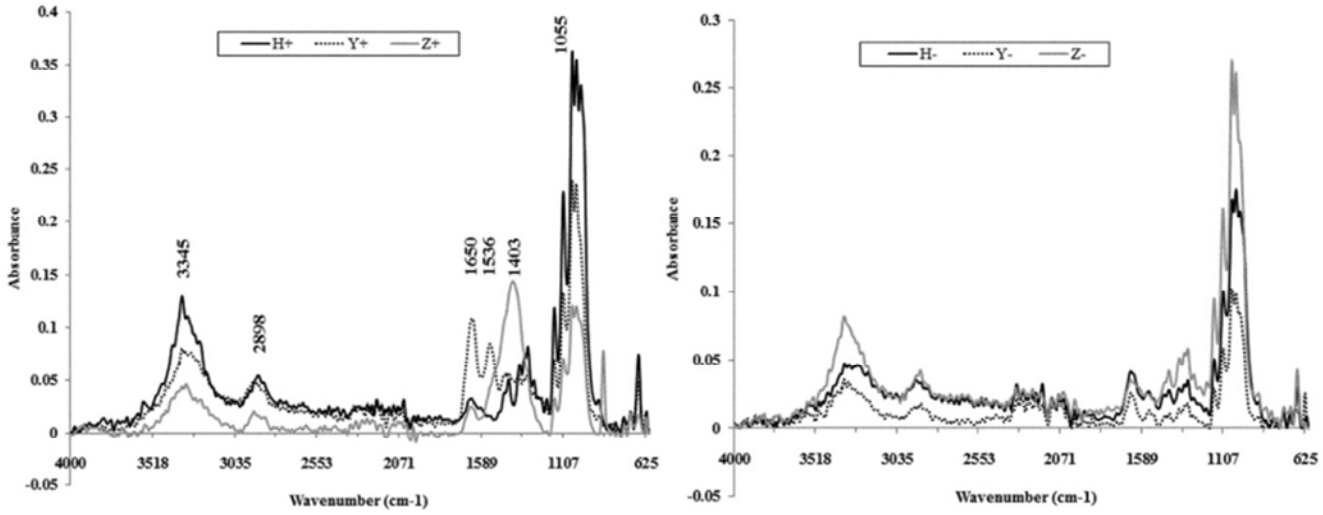

Figure 3. *An overlay of FTIR spectra of BNC.*

Table 1. *FTIR band assignments of BNC [11].*

Wave number (cm1)	Assignment
Around 3345	OH stretching of cellulose I
Around 2898	CH2 stretching
1644-1650	H-O-H bending vibration of absorbed water molecules
1543-1536	Protein amide II absorption
Around 1428	CH2 symmetrical bending or surface carboxylate groups
1314	CH2 wagging
1146-1160	Anti-symmetric bridge COC stretching
1107	C-O bond stretching
1050-1055	Ether COC functionalities and C-OH stretching vibration
870-900	Out of plane CH bending vibrations
665-670	Out of plane C-OH bending

The hydrogen bond intensity (HBI) of cellulose is closely related to the crystallinity. The ratio of absorbance bands at 3400 and 1320 cm^{-1} indicates the cellulose HBI. This ratio for BNC from medium H+ is around 1.09; while lower values were obtained for the other samples. The H+ medium BNC had a highly ordered form, which resulted in strong hydrogen bonds. The ratio between the heights of the bands at 1372 cm^{-1} and 2900 cm^{-1} determines total crystalline index (TCI) of cellulose. TCI data obtained by FTIR complete the crystallinity values obtained from XRD analysis. The highest and lowest TCI values of BNC were from media H+ (1.04) and Y+ (0.84), respectively. The absorption band at 1644-1650 cm^{-1} in BNC produced in medium Y+ was sharper than those of the others. This indicates that the moisture of BNC was higher than that of the BNC obtained under the other five conditions. Another difference in the spectra corresponds to the peak around 1403-1428 cm^{-1}; BNC harvested from Z+ medium had a very sharp absorption band, corresponding to CH_2 symmetrical bending, or surface carboxylate groups.

The absorption bands near 750 and 710 cm^{-1} are assigned to cellulose I_α and I_β, respectively. The ratio of cellulose I_α and I_β forms in BNC are given in Table 3. The highest crystallinity value was for BNC produced in medium H+ (76.2%), while its cellulose I_α component was the lowest.

The decrease in cellulose I_α showed the enhanced crystallization of cellulose I_β; which is presumably attributed to ethanol addition. Furthermore, medium constituents and moisture content of BNC can affect the aggregation of microfibrils and influence its crystallization. BNCs harvested from media Y+ and Z- had similar absorption assigned to –OH groups (3345 cm^{-1}). However, absorbed water and surface carboxylate groups of BNC produced in Y medium were more pronounced than those of BNC from medium Z-; which caused a decrease in crystallinity. There is a strong correlation between crystallite size and cellulose I_α contents.

BNC obtained from medium H+ was an exception from this rule. BNC from medium Z+ had a crystallite size of 6.5 nm and the lowest proportion of cellulose I_α (0.6).

3.2. X-Ray Diffraction Analysis (XRD)

The Cr (%) of cellulose and CrS were calculated from the diffractograms. The results of detailed calculations are summarized in Table 3.

Table 2. *Surface chemical composition of BNC.*

Medium	C (wt%)	N (wt%)	O (wt%)	O/C
H-	62.37	3.96	33.51	0.54
H+	59.88	2.74	37.06	0.62
Y-	66.92	8.29	24.29	0.36
Y+	42.80	3.16	52.21	1.22
Z-	59.17	2.11	38.16	0.64
Z+	37.17	2.77		

BNC from H and Y media had the highest and lowest Cr (%), respectively. It appears that the components from Y medium may interfere with the aggregation of BNC microfibrils, resulting in a lower Cr (%) [8, 12].

The BNC from Z medium had the lowest CrS size (6.5 nm); however, the BNC crystallite size did not differ greatly among media.

Table 3. *Crystallinity (%), crystallite size (nm) and mass fraction of cellulose Iα and I.*

Medium	Crystallinity (%)	Crystallite size (nm)	Mass fraction of cellulose Iα	Mass fraction of cellulose Iβ
H-	64.9	6.9	0.66	0.34
H+	76.2	6.9	0.48	0.52
Y-	53.9	6.7	0.68	0.32
Y+	53.2	6.9	0.71	0.29
Z-	55.9	6.8	0.71	0.29
Z+	60.6	6.5	0.60	0.40

3.3. Field-Emission Scanning Electron Microscopy (FE-SEM) and Energy Dispersive X-Ray Spectroscopy (EDS)

EDS analysis results for BNC are given in Table 2.

The O/C ratios of BNC derived from the media with ethanol addition were higher than those of BNC from the media without ethanol. This is confirmed by FTIR spectroscopy on BNC – i.e. the O-H stretching band at 3345 cm^{-1}. The lower O/C ratio of BNC from H+ medium (0.62),

compared with those of BNC from Z+ and Y+ media, is likely explained by its high crystallinity. The lowest BNC O/C ratio was derived from medium Y- (0.36).

This low O/C value was possibly caused by the purification process and medium-based organic impurities, such as N (weight 8.3%).

4. Conclusion

In this research, surface properties of bacterial nano-cellulose, produced from different culture media with and without ethanol, using spectroscopic methods and X-ray diffraction were studied.

Spectroscopic and x-ray diffraction analyses showed that BC from H and Y media had the highest and the lowest crystallinity, respectively. Cellulose I_α structure in BC was decreased in H+ medium, which presumably enhanced the crystallization of cellulose I_β and possibly influenced by the addition of ethanol. Moreover, ethanol addition increased O/C ratios of BNC samples significantly.

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