Some Properties of Bacterial Cellulose Produced by New Native Strain *Gluconacetobacter sp.* A06O2 Obtained from Turkish Vinegar

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ABSTRACT: The aim of the study was to isolate and identify an acetic acid bacterial strain having high cellulose yield and to investigate some physicochemical properties of bacterial cellulose (BC). Acetic acid bacteria were isolated by using 62 samples (vinegar, fruit, vegetable, and soil) from different region of Turkey. The cellulose production ability of 153 isolates was determined. A strain (A06O2) having high and stable cellulose yield was identified by biochemical tests and 16S rRNA gene sequencing and compared with type strain *Gluconacetobacter xylinus* NRRL B-759. Based on the results, strain A06O2 was named at the genus level as *Gluconacetobacter*, however, species level identification could not be made. Celluloses from both strains were purified to investigate the physicochemical properties such as thermal properties, solubility in various solvents, elemental composition, tensile properties, and surface properties by FTIR and SEM. The results showed that the cellulose samples of two bacterial strains differed in the physicochemical properties. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1823–1831, 2011

Key words: bacterial cellulose; *Gluconacetobacter*; SEM; FTIR; mechanical strength

INTRODUCTION

Cellulose is the most abundant biological macromolecule on the Earth with 180 billion tons per year produced and it is one of the oldest, natural, renewable, biodegradable, and biocompatible polymers.[1–3] It forms the basic structural matrix of the cell walls of nearly all plants, many fungi, and some algae. Several bacteria synthesis cellulose, including the genus of *Acetobacter, Agrobacterium, Pseudomonas, Rhizobium*, and *Sarcina*; however, only *Sarcina* is the genus of Gram positive bacteria in this field.[2,4,5] Although there are some microorganisms capable of synthesizing cellulose, *Acetobacter xylinum* is the only one that can synthesize it in sufficient abundance for industrial application.[6] The Gram negative bacterium, reclassified in 1997 as *Gluconacetobacter xylinus*,[7] synthesizes a network nanostructured formed by cellulose fibers with high mechanical resistance, resulting from their physical and chemical arrangement.[8]

The BC has been found to have a unique structure, composed of very thin fibers and displaying unique properties with a broad perspective for application in different fields, including composite membranes, medicine, artificial skins, blood vessels, paper, foods, textiles, and binding agents. The interest in cellulose produced by bacteria from surface cultures has increased steadily in recent years because of its potential for use in different fields.[3,5,9,10] Cellulose produced by *G. xylinus* is chemically pure, free of lignin and hemicelluloses in contrast to wood celluloses. It has high polymer crystallinity, high degree of polymerization, high purity, high water absorption and retaining capacity, high tensile strength, and strong biological adaptability that distinguish it from other forms of cellulose.[5,10]

In this study, some *Gluconacetobacter* sp. strains were isolated from various sources and determined their cellulose production. The strain showing the highest bacterial cellulose production was identified according to some physiological, biochemical and phylogenetic characteristics and differences. Then, the properties of BC were investigated.

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EXPERIMENTAL

Isolation materials, microorganisms, and culture medium

Total of 62 samples obtained from different region of Turkey were used as isolation materials. These included in home made grape vinegars (11 samples), apple vinegars (5 samples), vinegars from different fruits (11 samples), apple pulp (1 sample), various fruit and vegetables (15 samples), and vineyard soil (19 samples). Total of 153 bacterial strains were isolated and used for determination of the cellulose production ability. Reference strain *Gluconacetobacter xylinus* NRRL B-759 was obtained from USDA ARS Culture Collection. Hestrin-Schramm (HS) medium contained the following (per liter of deionized water): glucose 20 g, yeast extract 0.5 g, peptone 0.5 g, Na_2HPO_4 0.27 g, citric acid 0.15 g.[11]

Isolation procedures of acetic acid bacteria

Each sample (10 g or mL) was homogenized with 90 mL of 0.85% (w/v) sterile sodium chloride (Merck, Darmstadt, Germany) solution. Further decimal dilutions were prepared with the same diluent. Acetic acid bacteria were isolated from sterile dilutions of 0.1 mL of each of the samples by plating onto GYC agar (5% glucose, 1% yeast extract, 3% $CaCO_3$, 2% agar). Growth of fungi and Gram positive bacteria were inhibited by supplementing cycloheximide (actidione, 100 ppm, Merck) and bacitracin (0.2 U/mL) to the media, respectively. After incubation at 28°C for four to five days, isolation was conducted. Isolates were selected on the basis of colony and/or cellular morphologies. Gram (−) and catalase (+) strains were stored in HS medium with 20% glycerol at −20°C.[12–15]

Determination of cellulose production

Bacterial strains were inoculated into the sterile 200 mL HS medium of 1000-mL flasks. Cultivation was performed in static conditions at 28°C for seven days. After incubation, the pellets were treated with 0.1*N* NaOH solution at 80°C for 20 min to remove bacterial cells and medium components. The cellulose pellets were then rinsed three times in sterile deionized water. Purified cellulose was dried in a vacuum oven at 80°C for 8 h and weighed.[16]

Identification of strains

The bacterial strain that exhibited high cellulose production in HS Broth was identified up to species level with the following biochemical and physiological tests which are: (i) catalase and oxidase production, (ii) motility, (iii) growth on mannitol, (iv) ability to overoxidise ethanol, (v) nitrate reduction. Some biochemical tests such as growth on 3% (v/v) ethanol in the presence of 5% (v/v) acetic acid, requirement of acetic acid for growth, growth in presence of 30% (w/v) D-glucose and acid production from different carbon sources were also applied.[17]

16S rDNA phylogeny

DNA was isolated from cultures incubated at 28°C for three days by DNA isolation kit (Promega, USA). The 16S rDNA was amplified by PCR. Primers (Alpha DNA) 5′-end (16Sd, 5′-GCTGGCGGCATGCTTAA CACAT) and the 3′end (16Sr, 5′-GGAGGTGATC CAGCCGCAGGT) were used for amplification.[18] PCR amplification was carried out in 50-μL samples consisting of 5-μL bacterial extract and 45-μL amplification mixture, which contained 15 pmol of each primer (Alpha DNA) and PCR master mix (Fermentas Int. Inc., Canada). The reaction was performed in a GeneAmp PCR System (Hybaid, USA). For amplification of the 16S rDNA, the samples were incubated at 94°C for 5 min to denature the target DNA and then cycled 35 times at 94°C for 1 min, 58°C for 1 min and 72°C for 2 min. The samples were then incubated for 10 min at 72°C for final extension and were maintained at 4°C until tested.[19] DNA sequencing was performed with ABI 3100 Genetic Analyzer by external laboratory (Refgen Lab, Ankara, Turkey).

The 16S rDNA sequence of strain A06O2 was compared with all deposited nucleotide sequences in the GenBank database by Blast 2 options. Close relatives were chosen as the reference and aligned with each other using the Clustal W 1.8 program and the evolutionary history was inferred using the neighborhood-joining method.[20] The evolutionary distances were computed by the maximum likelihood method[21] and were in the units of number of base substitutions per site. All positions with gaps and missing data were eliminated from the dataset (complete deletion option). In the final dataset there were a total of 800 positions. Phylogenetic analyses were conducted in Molecular Evolutionary Genetics Analysis 4 (MEGA4).[22]

Gluconacetobacter xylinus NRRL B-759 was chosen as the reference from the closest genus *Gluconacetobacter* sp.

Production and purification of BC

The A06O2 strain was precultured at 28°C for 72 h without shaking in a 300-mL Roux flask containing 100 mL HS medium. Cellulose matrix was removed by an inoculation loop and medium containing bacterial cells was inoculated into the main culture medium (1 L) at 10% (v/v). The medium was poured

into the sterilized glass or stainless steel cooking tray with the size of 30 × 40 × 10 cm^3. After incubation at 28°C for 10 days, the jelly like cellulose matrix was taken from top of the medium. The matrix was boiled in 0.1*N* NaOH for 20 min and then was successively washed three times with deionized water. The final product was used as wet or dry for different purposes.[5,16]

Some properties of BC

Thermal analysis

The curves of the dried samples were recorded using Perkin–Elmer Pyris Diamond model thermal parametric analyzer (Beaconsfield, Buckinghamshire, HP91QA, England) with nitrogen a pure gas at a flow rate of 25 mL/min and at heating rate of 10°C/min. The percentage weight loss and derivative weight loss were recorded against temperature for all samples. The thermal behaviors of A06O2 and B-759 celluloses were compared by using thermal gravimetric analysis (TGA) curves.

Solubility tests

The solubility of cellulose was determined by using different solvents such as toluene, acetone, chloroform, benzene, carbontetrachloride and dimethyl formamide (Aldrich, USA). Solvents of cellulose were prepared by adding 0.1 g sample into 10 mL solvent, agitated at room temperature for 24 h and solubility of cellulose was observed.

Elemental analysis

Elemental composition (C, H, N, O) of cellulose was determined by elemental analyzer (Leco Chns-932, USA).

Fourier transform infrared spectroscopy analysis

The surface properties of the BC samples were carried out using a Fourier transform infrared spectrometer (Perkin–Elmer Spektrum 100 FTIR). Fourier transform infrared (FTIR) spectroscopy spectra were recorded in spectral range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}. Pellets of 10 mg of cellulosic samples were prepared by mixing with 300 mg of spectroscopic grade KBr. To elucidate some bands shielded by the broad OH bending mode of bound water, a FTIR spectrometer (BIO-RAD 175C) equipped with evacuation line was used. For this analysis, the pellets were prepared without KBr. Approximately 10 mg of the cellulose samples were pressed in a standard device using a pressure of 200 bar to produce pellets at a certain diameter. A total of 64 scans were taken per sample.[23–25]

Physical properties of BC

The tensile properties of the samples were tested on LLOYD-LR5K Plus tensile tester on which the ultimate tensile stress and breaking elongation values were determined. Tests were performed using a gauge length of 15 mm and at a crosshead speed of 150 mm/s. A preload of 0.1*N* was applied. The results are the averages of three measurements for each cellulose sample.

Scanning electron microscopy

Surface morphology of cellulose samples of two strains was monitored by using JEOL JSM 6060 Scanning Electron Microscope. The sample specimens were coated with gold (30 μm thick) in an automatic sputter coater (Polaron SC502). Accelerating potential was 10 kV. Photographs of representative areas of the sample were taken at 1000 and 5000 magnifications.

RESULTS AND DISCUSSION

Isolation, cellulose production, and identification of isolates

Bacterial growth was observed a few soil samples, therefore most of isolates were taken from vinegar samples. Colonies that formed after 72 h on GYC agar plates were uniformly round and white. The fresh cells in HS medium under microscope were straight rods, single or double. A total of 218 colonies with different morphology was isolated from the samples. According to their gram and catalase reactions, 65 strains were out of experiment. The bacterial strains (11 isolates) produced thick cellulose pellicles on the surface of the medium were selected for further analysis, however, during the experiments, only one strain (A06O2) appeared to be stable cellulose production. A maximum BC production of A06O2 (6.7 g dry weight/L BC) was achieved after seven-days incubation at 28°C in HS medium with 2% fructose under static conditions, while the production of B-759 was 2.0 g dry weight/L. BC amount of A06O2 was 3.35-fold higher than that of B-759. Son et al.[15] reported that BC yield of *Acetobacter* sp. V6 was found 4.16 g/L and 1.58 g/L in synthetic and complex media, respectively. As a consequence, A06O2 produced a considerable amount of BC.

The strain had some biochemical similarities to the reference strain such as gram reaction, oxidase activity, motility, oxidation of ethanol, and

TABLE I
Comparison of Physiological and Biochemical Characteristics of the Isolate A06O2 and *Gluconacetobacter xylinus* NRRL B-759

Tests	Isolate A06O2	B-759
Gram reaction	−	−
Oxidase	−	−
Catalase	+	+
Motility	+	+
Growth on YPM	+	−
Oxidation of ethanol	+	+
Nitrate reduction	−	−
Cellulose production	+	+
Growth in presence of 30% D-glucose	+	−
Growth on 3%(v/v) ethanol in the presence of 5% acetic acid	−	−
Requirement of acetic acid for growth	−	−
Acid formation from		
D-Glucose	+	+
D-Galactose	−	−
Glycerol	−	−
Fructose	+	+
Lactose	−	−
Maltose	−	−
Mannitol	−	−
Sucrose	−	−
Ethanol	−	−

production of acid from some carbon sources. The main differences between the two bacteria are growth in presence of 30% D-glucose and growth on YPM agar (Table I). Even though *G. hansenii, G. europaeus, G. xylinus* cannot grow in presence of 30% D-glucose, *G. intermedius* can grow in it. Morphological and biochemical characteristics of strain A06O2 such as growth in presence of 30% D-glucose and cellulose production show that the strain belongs to *G. intermedius*. On the other hand, it seems to like *G. xylinus* based on the growth on 3% (v/v) ethanol, in the presence of 5% acetic acid and cellulose production.[17] Therefore the strain A06O2 was not identified at the species level according to the physiological and biochemical analysis.

Amplified products of 16S rDNA contained 760 bp, and the sequences were submitted to BLAST search system. Although strain A06O2 was showed highest sequence similarity to *G. intermedius*, it had a difference of growth in 3% ethanol and 5% acetic acid from *G. intermedius*. Type strain *G. xylinus* NRRL B-759 also showed similar properties. The results are in agreement with Siever and Swings.[17] They were reported that *G. europaeus*, *G. xylinus, G. intermedius* and *G. oboendiens* have been accepted as very close relatives because they have more than 99% 16S rRNA gene sequence similarity.[17] Therefore, further molecular studies are necessary to establish exact identification of A06O2 at the species level. A phylogenetic tree based on the genotypic similarities is given in Figure 1.

The properties of BC

BC obtained from strain A06O2 investigated in terms of some properties and compared with BC of *G. xylinus* NRRL B-759.

Thermal analysis

Figure 2 shows the thermogravimetric/differential thermal analysis (TG/DTG) curves of BC samples. At the beginning of heating process (about 100°C), the water desorption caused the minor weight loss. The presence of water in the TG curves of both samples was observed. Then, the samples decomposed at temperatures above 280°C, and they were converted into volatiles, low molecular weight polysaccharides, and carbonaceous char. The initial degradation temperature of cellulose is an indication of the highest operating temperature for its application. Decomposition of cellulose started at 253.5°C and 235.8°C for A06O2 and B-759, respectively. Second decomposition process that involves depolymerization, dehydration, and the decomposition of glucosyl units followed by the formation of a charred residue occurred at temperatures higher than 200°C.[26] B-759 has a higher maximum decomposition temperature (320°C), obtained derivative weight loss curve, than that of A06O2 (300°C).

TG analysis was also measured the weight loss during heating. The first mass loss of B-759 cellulose is slightly higher than that of A06O2. The first low mass loss, occurring from 50 to 280°C, is due to dehydration by breaking of internal bonds. Physically adsorbed and hydrogen bond linked water molecules can be lost at that first stage.[27–29] However 50% weight loss was determined at 328.5°C and 325.6°C for A06O2 and B-759, respectively. Similar

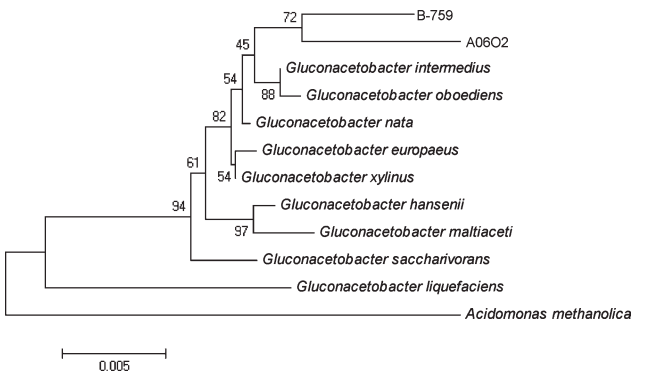


Figure 1 The phylogenetic tree of strain A06O2 based on 16S rDNA sequence similarities with selected reference sequences. The tree is drawn to scale, with branch lengths in same units as those of the evolutionary distances used to infer the phylogenetic tree. Bar represents 5 base substitutions per 100 nucleotides.

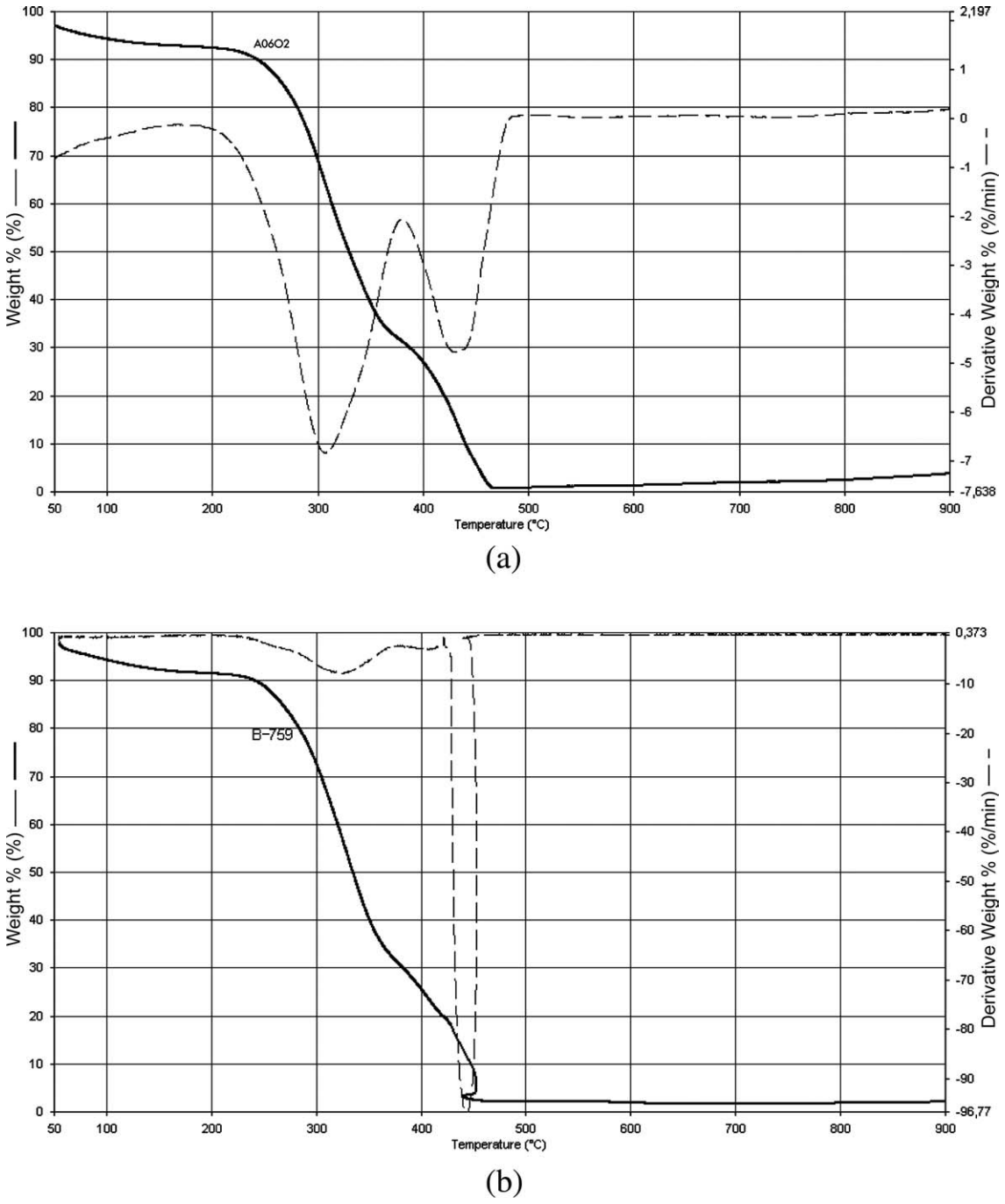


Figure 2 Thermal analysis diagram of two strains.

thermal behavior for native BC was reported by Liu et al.[30] On the other hand, thermal stability of different BC samples was found slightly lower[26,31] than that of our samples.

Solubility analysis

The solubility of cellulose depends on many factors, especially its structure, molecular weight and origin. At present, many scientific centers are interested in dissolving cellulose in inorganic and organic solvents.[32] However, cellulose is very stable and soluble in some solvents such as strong acids or strong hydrogen bonding solvent systems, usually amine-based. It was also reported that plant cellulose could be dissolved in some mixed solvents such as N_2O_4/DMF, SO_2/NH_3, DMSO/PF, LiCl/DMAc, NMMO, NaSCN/KSCN/LiSCN/H_2O. A LiCl/DMAc solvent system was used to dissolve BC (3 wt %) after an activation procedure.[33]

In our study, solubility features of the bacterial celluloses were determined by using different

TABLE II
Elemental Composition of BC Samples

Strains	Elemental composite (%)			
	C	H	N	S
B-759	43.02	6.23	0.10	0
A06O2	46.62	6.54	0.0	0

solvents like toluene, acetone, chloroform, benzene, carbontetrachloride, and dimethyl formamide. BC of *Gluconacetobacter* sp. A06O2 did not dissolve any solvents, whereas BC of *G. xylinus* NRRL B-759 dissolved only toluene. This insolubility has confirmed more H-bonding and more crystalline structure. The solubility can be explained with breaking of hydrogen bonds between polymer chains and increasing accessibility to intercrystallite structure. Owing to different strains and different molecular structures including crosslinking of BC is insoluble biomaterial. Compared with other natural biodegradable polymers like collagen and chitin, BC can be biopolymer with better properties for tissue engineering.[34]

Elemental analysis

The elemental analysis revealed the composition of carbon and hydrogen conforming to structure of cellulose. Therefore, the elemental composition of B-759 and A06O2 cellulose were calculated by the results from elemental analysis, as shown Table II. Elemental carbon content was determined 43.02% and 46.62% for B-759 and A06O2 cellulose, respectively. Moreover, these values are quite compatible with that of BC pellicles. These findings concurs with Yoon et al.[35] reported that elemental carbon and H contents of BC were 43.57% and 6.30%, respectively. It should be noted that the nitrogen belonging to B-759 is from the cell debris in the fibril of cellulose. It can be thought that A06O2 cellulose is more pure than B-759 cellulose.

FTIR analysis

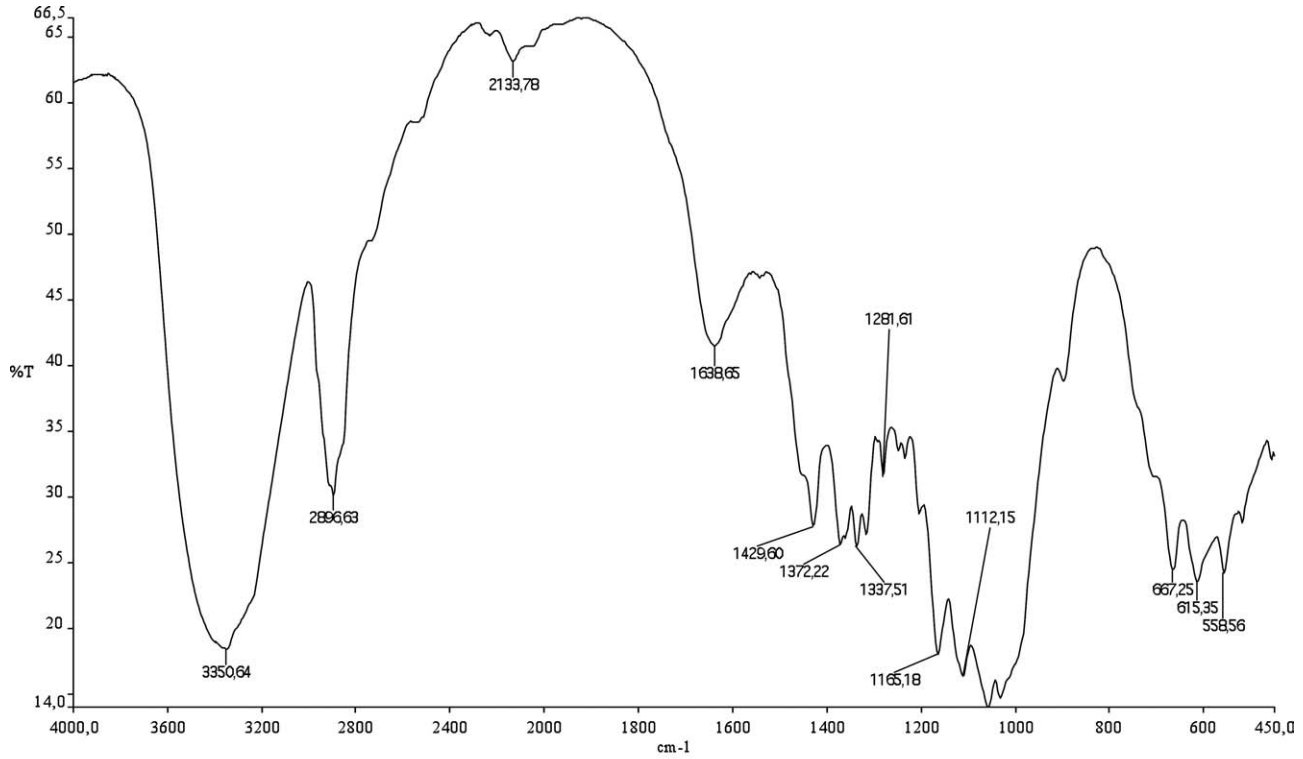
Cellulose is a biopolymer comprising of β-D-glucopyranose units linked together through β-1,4 glycosidic linkages, which can be characterized by FTIR spectroscopy. Figure 3 indicates FTIR spectra of A06O2 and B-759 celluloses. The FTIR spectra have characteristic bands for both A06O2 and B-759 cellulose. The major stretching peak associated with -OH groups on the glucose rings and water molecules take place between 3300 cm^{-1} and 3400 cm^{-1} in the different BC samples.[31,36] For A06O2 and B-759, a broad -OH stretching band was determined around 3350 cm^{-1}. The C—H stretching vibration bands of —CH_3 and —CH_2 groups are observed as a broad band centered at 2900 cm^{-1} whereas bending bands of these groups are observed at 1429, 1372, and 1333 cm^{-1},[37,38] however the intensities of bands at 2900 cm^{-1} and 1625 cm^{-1} were monitored for A06O2 and B-759 celluloses. The band intensity of A06O2 at 1625 cm^{-1} is weaker than that of B-759. It shows that structure of conjugate diene and aromatic are low in BC of A06O2. On the other hand, the band intensity at 2900 cm^{-1} is stronger for A06O2 than B-759 (Fig. 3). It shows that strain of A06O2 has aliphatic carbon groups more than B-759. These results have also confirmed that the bacterial celluloses obtained from two strains are different from each other in terms of compound. According to the spectra analysis, B-759 cellulose did not include a substantial amount protein. This was confirmed by elemental analysis which revealed the protein content of the sample as 0.1% N (w/w). Similar findings were reported by Bertocchi et al.[39] Although their BC samples contained less than 0.5% (w/w) protein, the presence of proteins were not observed from the FTIR spectra.

Physical properties

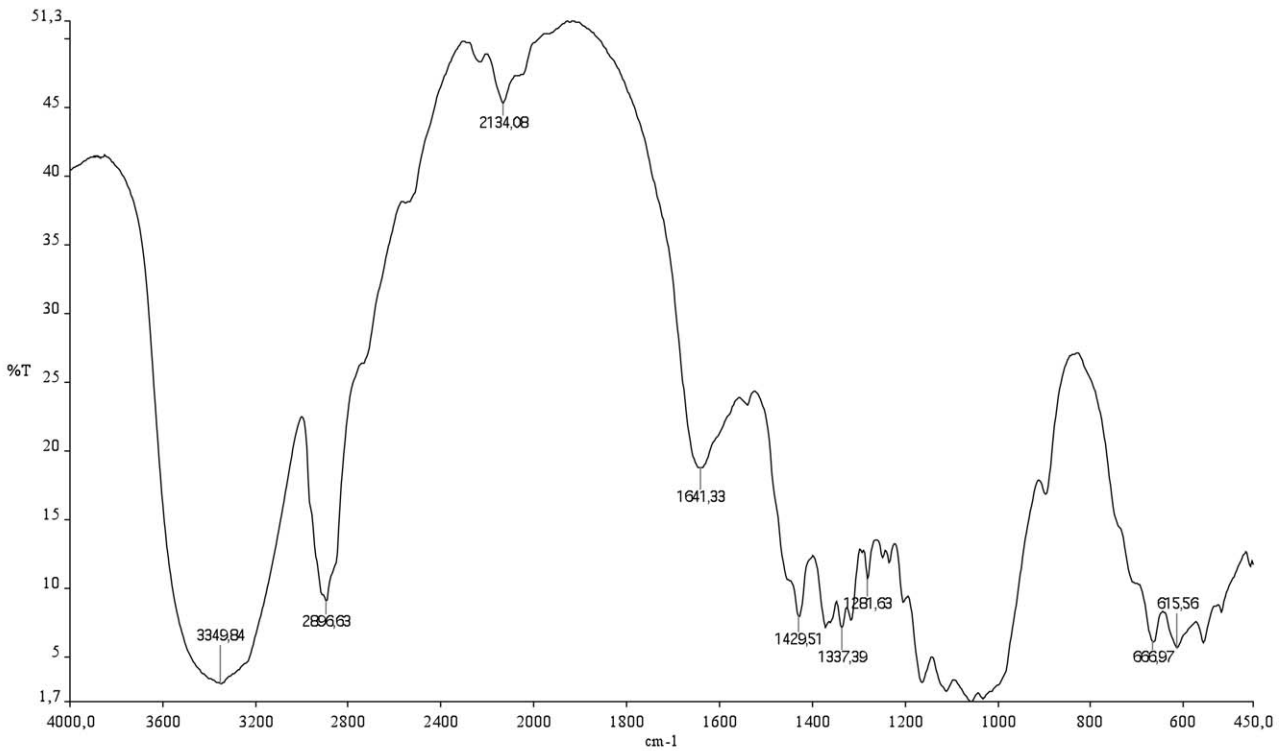
The stress–strain curves of both samples are given in Figure 4. The stress was low at the beginning of analysis, and then it rapidly increased. The tensile strength of B-759 reached to about 6.5*N*, while A06O2 had a 2.2*N* tensile strength. The results showed that ultimate tensile strengths of both strains were quite different. Low elongation at the rupture was similar for both BC samples. This result is similar to other studies about physical features of cellulose. In addition to that, ultimate tensile strength of paper increased when BC was added to paper dough.[40] Goelzer[8] reported that *G. xylinus* synthesizes a network nanostructured formed by cellulose fibers with high mechanical resistance, as a result of their physical and chemical arrangement. Changes in tensile strength and elongation with wetting may depend mainly on the number of the molecular chain ends in the amorphous region.[41]

Morphological observation of BC

SEM micrographs of BC from two strains at different magnifications are shown in Figure 5. SEM analysis indicated that two BC samples had different surface morphologies. Cellulose of B-759 had the typical microfibrillar, a mesh-like and porous appearance, while A06O2 cellulose also contained some fiber cluster at the vertical line. This could be the reason why the stress strength of A06O2 BC was lower than that of B-759 BC. Fibers of A06O2 represented a flat surface and had less pore space. The difference was confirmed by the determination of physical and chemical properties of cellulose such as FTIR



(a)



(b)

Figure 3 FTIR spectra of cellulose samples (a) A06O2; (b) B759.

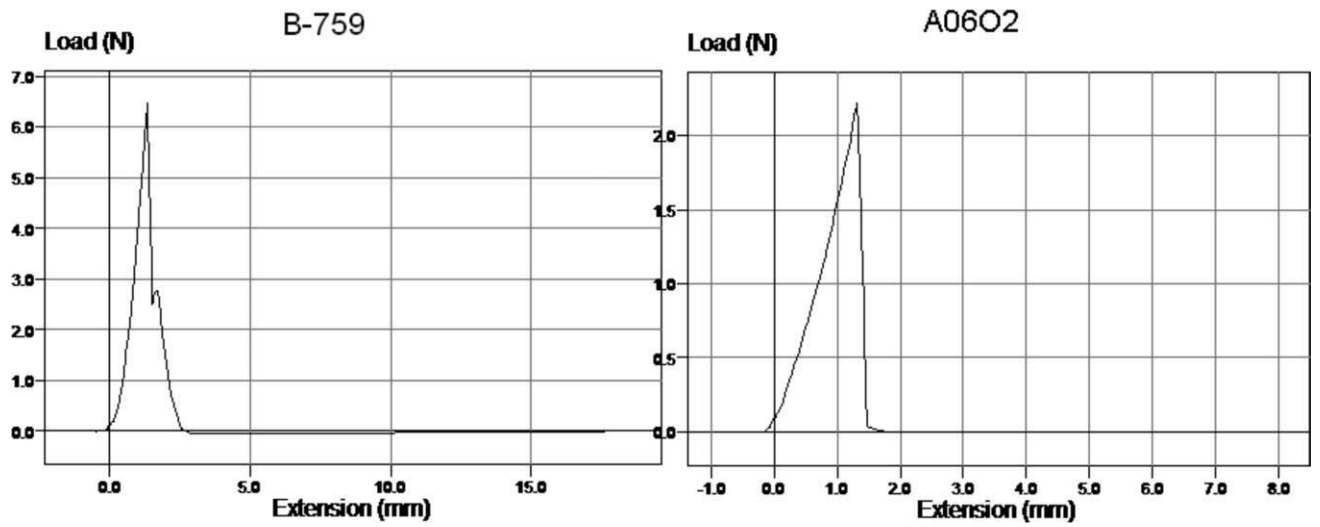


Figure 4 Strength-elongation diagram of cellulose samples.

spectra, solubility in organic solvents etc. BC is described as a well-organized three-dimensional porous network structure in the previous studies.[31] Some intact bacteria were also illustrated in Figure 5(A). BC was extruded from the pores of bacterial cellular membrane.

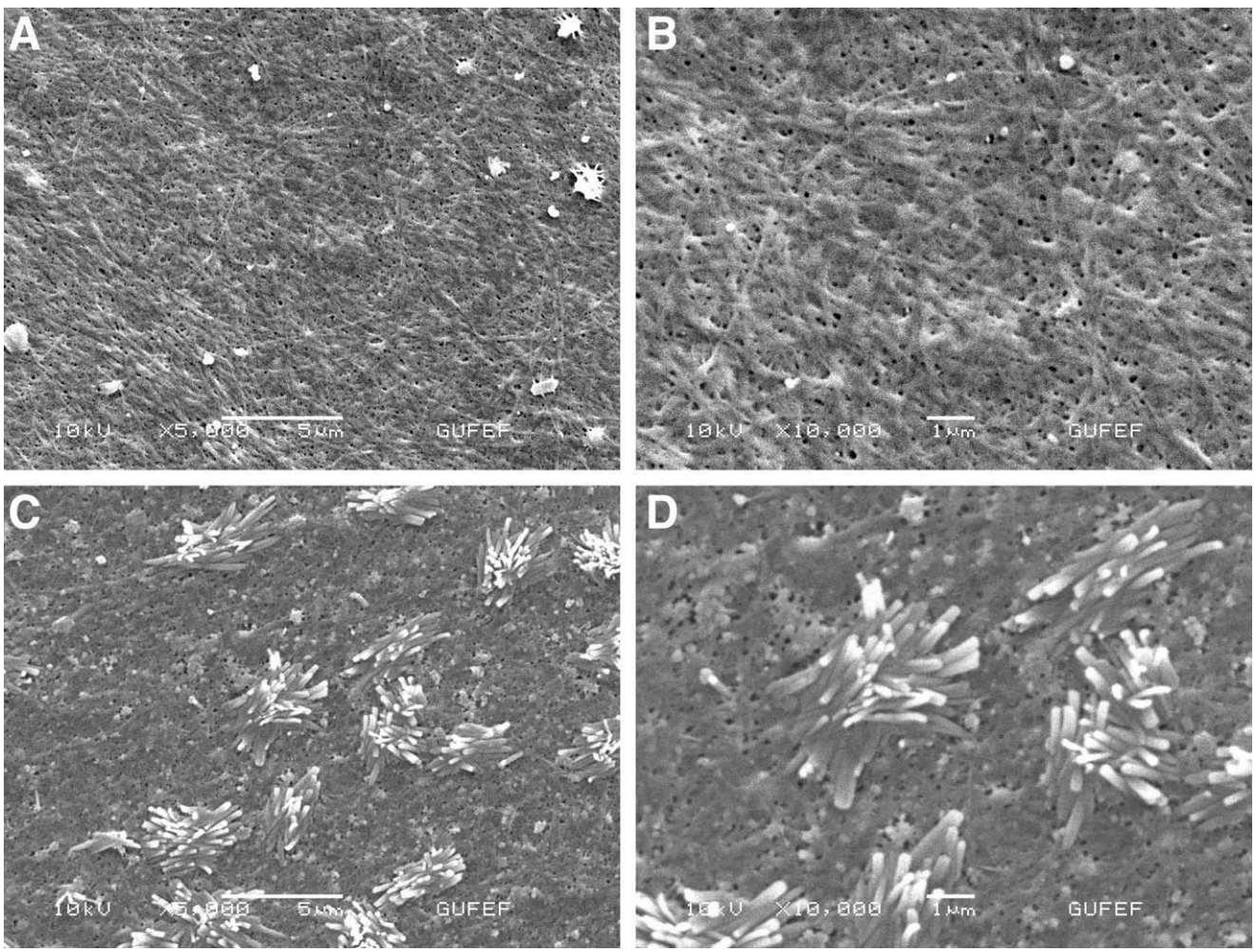


Figure 5 Morphology of bacterial cellulose samples at different magnifications. (A) Cellulose of B-759 at x5000; (B) Cellulose of B-759 at x10000; (C) Cellulose of A06O2 at x5000; (D) Cellulose of A06O2 at x10000.

References

1. Englehardt, J. Carbohyd Eur 1995, 12, 5.
2. Jonas, R.; Farah, L. F. Polym Degrad Stab 1998, 59, 101.
3. Wan, Y. Z.; Hong, L.; Jia, S. R.; Huang, Y.; Zhu, Y.; Wang, Y. L.; Jiang, H. J. Comp Sci Technol 2006, 66, 1825.
4. Cannon, R. E.; Anderson, S. M. Crit Rev Microbiol 1991, 17, 435.
5. Holmes, D. Bacterial Cellulose (PhD Thesis), Department of Chem and Process Engineering University of Canterbury Christchurch, New Zealand, 2004.
6. Brown, A. J Chem Soc 1986, 432.
7. Yamada, Y.; Hoshino, K. I.; Ishikawa, T. Biosci Biotechnol Biochem 1997, 61, 1244.
8. Goelzer, F. D. E.; Faria-Tischer, P. C. S.; Vitorino, J. C.; Sierakowski, M. R.; Tischer, C. A. Mater Sci Eng C 2008, 29, 546.
9. Fontana, J. D.; Souza, A. M.; Torriani, I. L.; Moreschi, J. C.; Gallotti, B. J.; Narciso, G. P.; Bichara, J. A.; Farah, L. F. Appl Biochem Biotechnol 1990, 24/25, 253.
10. Hong, F.; Qiu, K. Carbohyd Polym 2008, 72, 545.
11. Hestrin, S.; Schramm, M. Biochem J 1954, 58, 345.
12. Hwang, J. W.; Kook, Y. Y.; Hwang, J. K.; Pyun, Y. R.; Kim, Y. S. J Biosci Bioeng 1999, 88, 183.
13. Du Toit, W. J.; Lambrechts, M. G. Int J Food Microbiol 2002, 74, 57.
14. Son, H.; Heo, M.; Kim, Y.; Lee, S. Biotechnol Appl Biochem 2001, 33, 1.
15. Son, H. J.; Kim, H. G.; Kim, K. K.; Kim, H. S.; Kim, Y. G.; Lee, S. J Bioresource Technol 2003, 86, 215.
16. Bae, S. O.; Shoda, M. Appl Microbiol Biotechnol 2005, 67, 45.
17. Siever, M.; Swings, J. In Bergey's Manual of Systematic Bacteriology, 2nd ed., Staley, J. T.; Boone, D. R.; Brenner, J. D.; De Vos, P.; Garrity, G. M.; Goodfellow, M.; oel Krieg, N. R.; Rainey, F. A.; Schleifer, K. H., Eds.; Springer: New York, 2005; p 41–96.
18. Ruiz, A.; Poblet, M.; Mas, A.; Guillamón, J. M. Int J Syst Evol Microbiol 2000, 50, 1981.
19. González, Á.; Hierro, N.; Poblet, M.; Rozès, N.; Mas, A.; Guillamón, J. M. J Appl Microbiol 2004, 96, 853.
20. Saitou, N.; Nei, M. Mol Biol Evol 1987, 4, 406.
21. Tamura, K.; Nei, M.; Kumar, S. Proc Natl Acad Sci USA 2004, 101, 11030.
22. Tamura, K.; Dudley, J.; Nei, M.; Kumar, S. Mol Biol Evol 2007, 24, 1596.
23. Amiel, C.; Ariey, L.; Denis, C.; Pichon, P.; Travert, J. Lait 2001, 81, 249.
24. Oust, A.; Møretrø, T.; Kirschner, C.; Narvhus, J. A.; Kohler, A. J Microbiol Met 2004, 59, 149.
25. Oh, S. Y.; Yoo, D. I.; Shinb, Y.; Seo, G. Carbohyd Res 2005, 340, 417.
26. Barud, H. S.; de Araújo Júnior, A. M.; Santos, D. B.; de Assunção, R. M. N.; Meireles, C. S.; Cerqueira, D. A. Thermochim Acta 2008, 471, 61.
27. George, J.; Sajeevkumar, V. A.; Kumar, R.; Ramana, K. V.; Sabapathy, S. N.; Bawa, A. S. J Appl Polym Sci 2008, 108, 1845.
28. Laszkiewicz, B. J Appl Polym Sci 1998, 67, 1871.
29. Seifert, M.; Hesse, S.; Kabrelian, V.; Klemm, D. J Polym Sci: Part A: Polym Chem 2003, 42, 463.
30. Liu, C. F.; Sun, R. C.; Zhang, A. P.; Ren, J. L. Carb Pol 2007, 68, 17.
31. Cai, Z.; Kim, J. J Nanotechnol Eng Med 2010, 1, 021002-1.
32. Cheng, K. C.; Catchmark, J. M.; Demirci, A. J Biol Eng 2009, 3, 12.
33. Shen, X.; Ji, Y.; Wang, D.; Yang, Q. J Macromol Sci Part B 2010, 49, 1012.
34. Kim, J; Cai, Z.; Chen, Y. J Nanotechnol Eng Med 2010, 1, 011006-1.
35. Yoon, S. H.; Jin, H. J.; Kook, M. C.; Pyun, Y. R. Biomacromol 2006, 7, 1280.
36. Lojewska, J.; Miskowiec, P.; Lojewski, T.; Proniewicz, L. M. Polym Degrad Stab 2005, 88, 512.
37. Maréchal, Y.; Chanzy, H. J Mol Struct 2000, 523, 183.
38. Ali, M.; Emseley, A. M.; Herman, H.; Heywood, R. J Polymer 2001, 42, 2893.
39. Bertocchi, C.; Delneri, D.; Signore, S.; Weng, Z.; Bruschi, C. V. Biochim Biophys Acta 1997, 1336, 211.
40. Yamanaka, S.; Watanabe, K.; Kitamura, N.; Iguchi, M.; Mitsuhashi, S.; Nishi, Y.; Uryu, M. J Mater Sci 1989, 24, 3141.
41. Miyake, H.; Gotoh, Y.; Ohkoshi, Y.; Nagura, M. Polym J 2000, 32, 29.