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Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529

Bhavna V. Mohite, Satish V. Patil*

School of Life Sciences, North Maharashtra University, P.O. Box 80, Jalgaon 425001, MS, India

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ABSTRACT

The present study aims to investigate the physico mechanical, structural and thermal properties of the bacterial cellulose (BC) produced under shaking condition. Formation of characteristic cellulose sphere has been characterized by light and scanning electron microscopy. The purity of bacterial cellulose was confirmed by thin layer chromatography of hydrolyzed product and elemental analysis by Energy Dispersive Spectroscopy and Fourier transform infrared spectroscopy. High crystallinity bacterial cellulose (81%) composed by high Iα confirmed by X-ray diffraction and solid state C13 nuclear magnetic resonance spectroscopy. The *Z*-average particle size was 1.44 μm with high porosity of 181.81%. The water holding and absorption capacity was determined. Tensile strength reveals a Young's modulus of 15.71 ± 0.15 MPa and tensile strength of up to 14.94 MPa. The thermal behavior evaluated by thermogravimetry and differential scanning calorimetry shows the thermal stability of bacterial cellulose. The results demonstrated unique characteristics of bacterial cellulose produced at shaking condition.

1. Introduction

Cellulose is the most abundant biopolymer on earth produced in large quantity in nature which is most commonly harvested from trees and cotton (Engelhardt, 1995). Bacteria belong to genera Agrobacterium, Rhizobium, Pseudomonas, Sarcina and Acetobacter can also synthesize cellulose (Cannon & Anderson, 1991). Unlike cellulose from plants, bacterial cellulose (BC) is chemically pure and free of lignin and hemi-cellulose. Plant-derived cellulose and BC have the same chemical composition but different structures and physical properties (Cannon & Anderson, 1991). As opposed to cotton and paper, where the purification of the cellulose product decreased the chain length, bacterial cellulose does not require remedial processing to remove unwanted polymers and contaminants (e.g. lignin, hemicellulose) and therefore, retains a greater degree of polymerization (Nishi et al., 1990). This fact gives bacterial cellulose superior unidirectional strength. *Acetobacter xylinum* produces two forms of cellulose: (i) cellulose I, the ribbon-like polymer, and (ii) cellulose II, which is thermodynamically more stable amorphous form of polymer (Brown, 1989). Cellulose I composed of parallel β, 1-4 glucan chains which are arranged uniaxially with van der Waals forces whereas β, 1-4 glucan chains of cellulose II are arranged in random manner. They are mostly antiparallel and with large number of hydrogen bonds that results in more stable form (Yu & Atalla, 1996). In nature, Cellulose I structure is found in allomorphic forms I_α or I_β, depending on the arrangement of the chains between each other. Cellulose belonging to plant cell walls shows higher percentage of I_β structure, compared with cellulose from algae and bacteria, that shows higher percentage of I_α structure, which seems to be a less stable displacement (Scionti, 2010). Normally *A. xylinum* cellulose displays characteristic of highly crystalline, I_α rich cellulose (VanderHart & Atalla, 1984). Microfibrillar structure of BC is responsible for most of its properties such as high tensile strength, high crystallinity index and higher degree of polymerization.

Due to specific properties such as the three dimensional nanomeric structure, unique physical, mechanical and thermal properties together with its higher purity BC has been commercialized as high end products like health food, high strength papers, audio speakers, filtration membranes, wound dressing materials, artificial skin, artificial blood vessels, and other biomedical devices (Czaja, Young, & Kawecki, 2007; Eming, Smola, & Kreig, 2002; Fontana et al., 1990; Okiyama, Motoki, & Yamanaka, 1993).

After 1990s research has been started for bacterial cellulose production focusing on its production, structural features, properties and applications. Suitable cultivation conditions (Bae & Shoda, 2004) and culture parameters (Jung, Park, & Chang, 2005; Son, Chung, Lee, & Kim, 2002) improve cellulose production. Bacterial cellulose has been produced under static or submerged shaking conditions (Chawla, Bajaj, Survase, & Singhal, 2009). In static

* Corresponding author. Tel.: +91 0257 2257421–25; fax: +91 257 2258403.
E-mail address: satish.patil7@gmail.com (S.V. Patil).

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culture systems, BC production is low (Park, Jung, & Park, 2003) and requires more area, work force, and time, results into expensive production (Sani & Dahman, 2009). Shaking culture gives better BC production by improvement of cultural conditions (Son et al., 2003). Besides there are some problems with shaking culture like conversion into cellulose non producing mutants, many researchers suggested agitated culture as most economical for mass production (Ross, Mayer, & Benzimann, 1991; Yoshinaga, Tonouchi, & Watanabe, 1997). In our previous study we have optimized the production media and culture conditions for high BC production under shaking conditions (Mohite, Kamalja, & Patil, 2012; Mohite, Salunke, & Patil, 2013). The understanding of properties of BC will provide more opportunities in its widespread utilization. Hence it is necessary to study the characteristic properties of BC under shaking conditions. The characterization of BC produced under shaking condition has been previously tried with only few workers focusing on physical (Suwannapinunt, Burakorn, & Thaenthanee, 2007), Structural (Czaja, Romanovicz, & Brown, 2004), thermal and mechanical (Cheng, Catchmark, & Demirci, 2009) properties. It has been revealed that the characteristics of BC could be affected by many factors, such as media, fermentation modes and carbon sources. In our investigation we have studied the synthesis and structural characteristics of bacterial cellulose produced at shaking condition by *Gluconoacetobacter hansenii*. Morphological differences between the cellulose produced by static and agitated cultures contribute to varying degrees of crystallinity, different crystalline size and Iα cellulose content (Chawla et al., 2009). We try to explore the characteristics of bacterial cellulose produced at shaking condition such as the purity, structural, physical, mechanical and thermal properties along with determination of water absorbance and holding capacity, particle size, porosity and purity analysis. To the best of our knowledge this is one of the rare reports summarizing majority of the characteristics of BC produced under agitating conditions.

2. Materials and methods

2.1. Microorganism, production and purification of BC

G. hansenii (NCIM 2529) from National collection of industrial microorganisms, National Chemical Laboratory, Pune, India was used in this study. The *G. hansenii* from Hestrin Schramm (HS) agar slants inoculated into HS broth (pH 6.0). The flasks were incubated at 30 °C for 2 days at 120 rpm in an orbital shaking incubator. This is used as 5% (v/v) inoculum. In Previous study the production medium was statistically optimized (Mohite et al., 2012) and used here for production having composition as follows (in gram per liter): sucrose, 28.1; KNO_3, 5; Na_2HPO_4, 0.1 g; $CaCl_2$, 12.6; $MgSO_4$, 1 g with reaction parameters as: pH 3.88 temperature, 25 °C; incubation time, 5 days; agitation speed, 170 rpm. After incubation of 5 days, the produced beads of BC were separated by filtration and rinsed with distilled water to remove excess media, and then immediately boiled (at 90 °C) in 0.1 M NaOH solution for 30 min to remove the cells and medium embedded in the cellulose material. After boiling, the beads of BC were purified by extensive washing in distilled water at room temperature until the pH of the water became neutral. This obtained purified cellulose was lyophilized in powder form.

2.2. Characterization

2.2.1. Purity of bacterial cellulose

2.2.1.1. Thin layer chromatography (TLC) of hydrolysis product of bacterial cellulose. BC was treated with 8 N H_2SO_4 for 2 h. After hydrolysis of the polymer, the glucose monomers were detected by TLC with solvent system of ethyl acetate:propanol (65:35) with standard glucose as control and iodine was used as detecting agent (Zogaj et al., 2003).

2.2.1.2. Acetan (water soluble polysaccharide) detection. The harvested broth was centrifuged and bacterial cellulose beads were separated. The supernatant was mixed with ethanol in 1:3 proportions. The formation of precipitate of acetan was checked (Ishida, Sugano, Nakai, & Shoda, 2002).

2.2.2. Field emission scanning electron microscopy (FE-SEM) and Energy Dispersive Spectroscopy (EDS)

Scanning electron microscopy was used to determine the morphology and surface topography of the BC fibers, micrographs of the gold-coated samples of freeze dried BC were taken with a Field-emission scanning electron microscope (Hitachi S-4800, Japan). The element analysis was done by *Energy Dispersive Spectroscopy* (Brucker, Germany).

2.2.3. Fourier transform infrared spectroscopy (FTIR)

The BC produced by *G. hansenii* NCIM 2529 from our studies was mixed well with potassium bromide (KBr) powder and pressed into a small tablet. Fourier transform infrared spectroscopy (FT-IR) spectrum was recorded using a Shimadzu spectrometer (8400, Japan) in the transmittance mode with a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}.

2.2.4. X-ray diffraction (XRD)

The freeze-dried samples were ground into powder for x-ray diffractometry analysis. The diffractograms were recorded at room temperature (Model D8 Advance, Brucker, corporation, Germany) using Ni-filtered Kα Cu X-ray radiation (λ = 1.54 Å). The operating voltage and current were 40 kV and 40 mA, respectively. Data were collected in reflection mode in the 10–40 2θ-range with a step of 0.02° 2θ intervals. The scans proceeded at 56.58 s per step. DIFRAC.EVA Suite software (Germany) was used to process the diffraction pattern and to calculate the crystallinity of BC.

2.2.5. Solid state C13 nuclear magnetic resonance (NMR) analysis

CP/MAS C13 NMR analysis was conducted on lyophilized sample using a spectrometer BRUKER DSX-300 solid state NMR spectrometer at −70 °C. The details of the instrument analysis are as follows: Magnetic field: 7.04 T, Platform: Linux & X-Winnmr, Proton freq.: 300.013 MHz, Carbon freq.: 75.47 MHz. Samples were packed in 7 mm ZrO_2 rotors and spun at 5 kHz. The experiments used recycle delays of 5 s, and each spectrum is the sum of 128 scans.

2.2.6. Mechanical analysis

The mechanical properties of the BC pellicles were analyzed through uniaxial tensile tests, using a testing machine Shimadzu AG-100KN, equipped with a biobath system containing distilled water, and a temperature-controlling system. The specimens were cut in a rectangular shape using a paper cutting machine, producing samples with dimensions of 1 cm × 5 cm individual sample thickness. A Bliss classic+ digital thickness indicator was used to measure the thickness of each specimen. Two samples were cut per pellicle, and three pellicles were used in each experimental session and average value was calculated f the three samples.

Using the Winsoft Tensile for Compression testing, connected to the Shimadzu machine, it was sufficient to insert the dimensions of the samples before the beginning of the experiments, and the software calculated automatically the values of stress and strain during the test. The Young's modulus was calculated using the same software, selecting manually the section of the stress–strain curves where it was required to measure it: the modulus was not measured