



High-performance green nanocomposites using aligned bacterial cellulose and soy protein



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ABSTRACT

Maximum potential of any fibers as reinforcing agent in composite materials can be obtained through their preferential alignment. In this study, a facile method has been developed to fabricate high-performance green composites based on aligned bacterial cellulose (BC) as reinforcing agent and soy protein as bio-resin. Aligned BC nanofibrils were produced by cultivating it inside polydimethylsiloxane (PDMS) tubes followed by an optimum wet-stretching. The curved geometry of the inner tube surface provided the bacteria a preferred direction for growth. Further improvement in alignment was obtained with optimized stretching of the tube-shaped BC (TBC) at a crosshead speed of 0.03 mm/min and draw ratio of 1.20. Stretched TBC showed superior mechanical properties in comparison to the conventionally-grown BC pellicle. Stretched TBC resulted in higher tensile strength (230% increase), modulus (330% increase), and orientation indices (135% increase) compared to the conventional BC pellicle. Soy protein isolate (SPI)-based fully green composites reinforced with TBC were prepared by a vacuum-assisted resin impregnation process, and the same stretching method was applied to the TBC-SPI gel. The stretched and cured composites showed significant improvements in the tensile strength (from 40 MPa to 150 MPa) and modulus (from 2 GPa to 8 GPa) in comparison to the conventional BC reinforced composite because of the enhanced alignment of BC nanofibrils.

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1. Introduction

Extracellular or bacterial cellulose (BC) synthesized by nonpathogenic aerobic bacteria in a culture medium containing carbon and nitrogen sources can be promising biodegradable nanofibers for composite materials because of their excellent mechanical properties [1–3]. Although BC is chemically identical to plant-based cellulose, it exists in extremely pure form and does not contain any hemicellulose, lignin, pectin or wax materials [4]. In addition, it has higher crystallinity, specific surface area, and mechanical properties compared to the plant-based cellulose [2,5]. It also has other unique properties such as low density and thermal expansion along with high optical transparency [1,6–8]. As a result, BC has received increased emphasis on its utilization as a reinforcement in composites to manufacture the next generation of advanced green structures [6,9,10].

The mechanical properties of BC are controlled by several factors including crystallinity, degree of polymerization, specific surface

area, and orientation [11,12]. A number of efforts have been made to obtain improved quality of BC by maximizing some of these factors through the adjustment of carbon and nitrogen sources in the culture medium. However, the orientation of nanofibrils cannot be easily manipulated once the random orientation is achieved due to the free movement of the bacteria in a conventional cultivation setup [12]. It is well-known that a composite with aligned fibers results in better mechanical performance in comparison to the same composite where proper alignment of fibers is lacking [13–15]. Similarly, BC nanofibrils aligned in a preferred direction can be expected to enhance the mechanical properties of the composite in that direction. Earlier, it has been shown that BC can be shaped suitably for various applications during biosynthesis [16]. One such interesting shape, tube-shaped BC (TBC), was produced by a matrix reservoir cultivation vessel technique for the biomedical purpose [1]. Later, Putra et al. developed a facile and scalable technique to produce TBC using a silicone tube [16]. They observed that TBC had aligned nanofibrils in the structure. However, neither the mechanical properties of such aligned TBC have been analyzed nor have they been used as reinforcement in any composite. In addition, further alignment in TBC should be possible

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by using a controlled wet-stretching process with optimal cross-head speed and draw ratio. Although conventional BC pellicle has been investigated as a reinforcement in composite materials [6,11,12], no study has yet been conducted to characterize the behavior and properties of TBC as a reinforcement in composite materials.

Plant proteins, extracted from defatted seeds and grains, have been utilized as sustainable resins for eco-friendly green composites [17–19]. Soy protein is one of the most abundant renewable resins and enjoys several other advantages such as being inexpensive, biodegradable, and environmental-friendly [17,20]. The resins developed from soy protein have desired polar groups to form hydrogen bonds with cellulose and proper hydrophilicity to wet the cellulose when fabricated into green composites [21].

In the present study, a simple and inexpensive process for the development of high-performance green composites is presented based on the aligned TBC and soy protein isolate (SPI) resin. The first half of this paper focuses on the development and production of BC with excellent nanofibrillar alignment using a combination of tube-grown method and wet-stretching process where tube dimensions, crosshead speed and draw ratio during stretching were controlled for optimal performance. Stretched TBC was analyzed to determine the effect of the process on morphology, alignment, and mechanical properties. The second half presents the preparation and characterization of TBC reinforced SPI (TBC-SPI) composites. To prepare TBC-SPI composites, SPI resin was impregnated into TBC via a vacuum-assisted resin impregnation method. The same stretching process used for TBC was applied to TBC specimens impregnated with the SPI resin before curing. Effects of wet-stretching on the mechanical properties and morphology of cured composites were investigated using tensile properties measurement and field-emission scanning electron microscopy (FESEM), respectively.

2. Experimental details

2.1. Materials

Acetobacter xylinum (*A. xylinum*) strain, ATCC 23769 was purchased from American Type Culture Collection (Manassas, VA). D-mannitol, yeast extract, tryptone, and acetic acid were purchased from Fisher Scientific (Pittsburgh, PA). Sodium hydroxide (NaOH) pellets and ammonium nitrate (NH_4NO_3 , $\geq 98\%$ purity) were purchased from Sigma-Aldrich Chemical Co. (Allentown, PA). SPI was provided free by Archer Daniels Midland Co. (Decatur, IL). High-temperature polydimethylsiloxane (PDMS) rubber tubes of various dimensions, standard wall adapters, and caps were purchased from McMaster Carr (Syracuse, NY).

2.2. Preparation of TBC hydrogel

A. xylinum strain was maintained on agar plate containing 2.5% (w/v) D-mannitol, 0.5% (w/v) yeast extract and 0.5% (w/v) tryptone, and 2.0% (w/v) agar. The strain from the agar plate was transferred into a sterilized culture medium containing the same amount of D-mannitol, yeast extract and tryptone to produce a seed culture. The seed culture and autoclaved culture medium were then mixed at a ratio of 1:8 and maintained at $\text{pH } 5.0 \pm 0.2$. PDMS tubes (Inner diameter, ID: 6.3 mm and Outer diameter, OD: 7.9 mm) were used as molds after autoclaving at 120°C for 15 min for the production of TBC. The culture medium containing bacteria was poured into the tubes and incubated at 29°C for 10 days with an angular position of 45° in a static state. Fig. 1 shows the illustration of the setup to produce the TBC hydrogel. TBC hydrogel produced at the interface of the culture medium and the inner surface of the tube was taken out and washed with 1 N NaOH solution at 90°C for 15 min. This

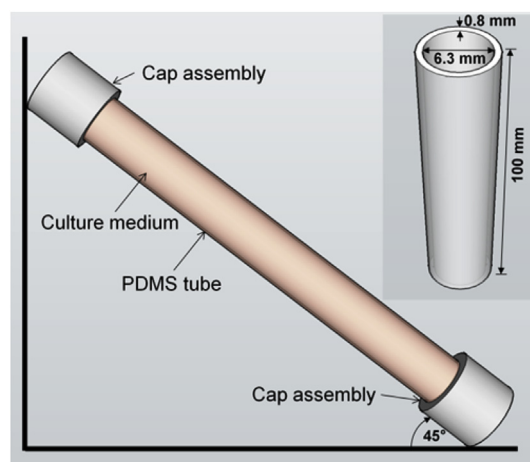


Fig. 1. Illustration of the experimental setup used to obtain tube-shaped BC (TBC). Inset shows the dimensions of the PDMS tube.

was followed by washing with deionized (DI) water at room temperature (RT) to remove the dead bacteria and chemicals from the culture medium. The purified hydrogel was stored in DI water at 4°C .

2.3. Wet-stretching of TBC hydrogel

The optimum stretching of TBC hydrogel was performed using a universal tensile testing machine (Instron 5566, Canton, MA) in a conditioning room ($65 \pm 2\%$ RH and $21 \pm 1^\circ\text{C}$). TBC hydrogel with a gauge length of 30 mm was stretched at a controlled crosshead speed of 0.03 mm/min (engineering strain rate of 10^{-3} min^{-1}) until the strain reached 20% or a corresponding draw ratio of 1.2. Stress-strain plots, based on engineering stress and strain values, were recorded to determine optimum stretching for the TBC hydrogel. The stretching was stopped at that time, and the stretched sheet was kept in between two PVDF filter papers (Durapore[®] membrane filters, Filter type: 0.45 μm , Merck Millipore Ltd., Ireland) and cardboards for drying at 50°C for about 8 h. These specimens are referred to as 'Stretched TBC' in this study. Un-stretched TBC (without any stretching) specimens were prepared using the same drying process and are referred to as 'Control TBC'.

2.4. Preparation of TBC-SPI composites

TBC-SPI composites were prepared using a water-based approach. At first, SPI powder with 10 wt% sorbitol as a plasticizer was homogenized in DI water at a ratio of 1:16 (w/v) using a magnetic stirrer for 15 min at RT and further stirred for 20 min at 80°C by adjusting pH at 10.5 ± 0.2 using a 1 N NaOH. TBC hydrogel was then impregnated with the SPI resin under a vacuum of -0.1 MPa at RT for 30 min in a vacuum oven. After the resin impregnation, specimens were taken out from the vacuum oven, and excess resin from all surfaces was removed using filter paper. TBC-SPI composite gel specimens were dried at 50°C for about 8 h and cured using a hydraulic hot press (Carver Inc., Wabash, IN) at 120°C for 3 min under a pressure of 8.5 MPa. These specimens are referred to as 'Control composites'.

The same stretching process mentioned in 'Wet-stretching of TBC hydrogel' section was performed on TBC specimens impregnated with SPI, prior to curing the resin. After stretching, the specimens were dried and cured using the similar process mentioned for the control TBC-SPI composites. These composites are referred to as 'Stretched composites'. All specimens were

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