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Production of bacterial cellulose using different carbon sources and culture media

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ABSTRACT

In this work, the effects of carbon sources and culture media on the production and structural properties of bacterial cellulose (BC) have been studied. BC nanofibers were synthesized using *Gluconacetobacter xylinus* strain PTCC 1734. Media used were Hestrin–Schramm (H), Yamanaka (Y), and Zhou (Z). Five different carbon sources, namely date syrup, glucose, mannitol, sucrose, and food-grade sucrose were used in these media. All the produced BC pellicles were characterized in terms of dry weight production, biomass yield, thermal stability, crystallinity and morphology by thermogravimetric analysis (TGA), x-ray diffraction (XRD), and field emission scanning electron microscopy (FE-SEM). The obtained results showed that mannitol lead to the highest yield, followed by sucrose. The highest production efficiency of mannitol might be due to the nitrogen source, which plays an important role. The maximum improvement on the thermal stability of the composites was achieved when mannitol was used in H medium. In addition, the crystallinity was higher in BC formed in H medium compared to other media. FE-SEM micrographs illustrated that the BC pellicles, synthesized in the culture media H and Z, were stable, unlike those in medium Y that were unstable. The micrographs of BC produced in media containing mannitol and sucrose provided evidence of the strong interfacial adhesion between the BC fibers without noticeable aggregates.

1. Introduction

Cellulose is one of the most common carbohydrate polymers found in the world and has been widely studied during the past decades (Lin, Lopez-Sanchez, Li, & Li, 2014). Although it is a major structural biopolymer of all plants, various bacteria are also able to produce it (Tanskul, Amornthatree, & Jaturonlak, 2013). Cellulose from plants is normally mixed with lignin and hemicelluloses, but cellulose from bacteria or bacterial cellulose (BC) contains sets of parallel chains composed of β-D-glucopyranose units interlinked by intermolecular hydrogen bonds, which is identical in chemical composition to plant cellulose (Dahman, Jayasuriya, & Kalis, 2010). BC displays many unusual physicochemical and mechanical properties, including higher purity, higher crystallinity, higher degree of polymerization (Ashori, Sheykhnazari, Tabarsa, Shakeri, & Golalipour, 2012), higher water absorbing and holding capacity (Saibuatong & Phisalaphong, 2010), higher tensile strength (Castro et al., 2011) and stronger biological adaptability (Ul-Islam, Khan, & Park, 2012). 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 (Shah, Ul-Islam, Khattak, & Park, 2013; Lin et al., 2014).

In recent years, much interest has developed in producing BC on a large commercial scale (Czaja, Young, Kawechi, & Brown, 2007; Castro et al., 2011). However, compared with other popular commercial organic products, BC is still expensive, therefore, its use is limited (Sheykhnazari, Tabarsa, Ashori, Shakeri, & Golalipour, 2011). There have been many reports of cellulose being produced by both Gram negative bacteria such as *Gluconacetobacter xylinus*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Azotobacter*, *Pseudomonas*, and *Rhizobium*, and Gram positive bacteria such as *Sarcina* (Tanskul et al., 2013). Among the mentioned genera, *G. xylinus* is one of the most commonly studied sources of BC (Nguyen, Flanagan, Gidley, & Dykes, 2008). It has been used for producing commercial quantities of cellulose and has become the most popular strain so far for application not only as a food component but also for drug-delivery systems (Tanskul et al., 2013), wound

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healing and tissue regeneration (Czaja et al., 2007), flexible display screens (Nakagaito, Nogi, & Yano, 2010), composite reinforced, electronic paper (Jonas & Farah, 1998), and so on. However, it is difficult to obtain a high productivity using this bacterium in a large-scale fermentation system due to its low yield under agitated conditions. It is important to develop methods to produce BC at the lowest possible cost. The significant factors that many researchers have investigated are the optimal medium, the culture conditions and their interaction effects. Additionally, determining growth conditions that produce high amounts of cellulose is necessary to complete further research using BC as reinforcement for biodegradable polymers as well as understanding any effects such conditions have on the basic materials' morphology and properties (Ruka, Simon, & Dean, 2012). There have been several reports of different media used in the literature, as well as various carbon sources (El-Saied, El-Diwany, Basta, Atwa, & El-Ghwas, 2008; Jung et al., 2010; Ruka et al., 2012). Some authors have reported that the structure of cellulose is not affected by changing the carbon or nitrogen source (Mikkelsen, Flanagan, Dykes, & Gidley, 2009), whereas others have reported differences. El-Saied et al. (2008) reported that a medium containing corn steep liquor and molasses medium resulted in a higher degree of crystallization over carbon and nitrogen sources such as glucose, mannitol, yeast extract and peptone, whereas Jung et al. (2010) reported a decrease in crystallinity in molasses medium compared to a complex medium control.

This work aimed at studying the effects of various culture media and carbon sources in order to formulate a general, simple and inexpensive medium to produce BC. More common carbon sources such as glucose, mannitol, sucrose, date syrup and food-grade sucrose were used in this study. Carbon sources were compared in terms of biomass and production yield, and the morphology and structure of the BC obtained were studied. In this context, BC nanofibers produced in the selected media were characterized by means of x-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM) and thermogravimetric analysis (TGA).

2. Experimental

2.1. Bacterial strain

The organism used was a native strain of *G. xylinus* obtained from the Persian Type Culture Collection (PTCC 1734), Iran. The strain was cultured on glucose yeast extract (GYE) agar containing 100 g D-glucose, 10 g yeast extract, 5 g peptone, 20 g $CaCO_3$, 25 g agar per liter at 28 °C for 3 days. Working cultures were routinely prepared on GYE and stored at 4 °C until use.

2.2. Media and carbon sources

Three different types of media that have been previously reported to have optimized concentrations and are used to cultivate *G. xylinus* were selected from the literature and modified for the present study. Media used were Hestrin–Schramm (H), Yamanaka (Y) and Zhou (Z). The chemical compositions of the media are described in Table 1. Date syrup, glucose, mannitol, sucrose, and food-grade sucrose were used as different carbon sources in these media. All the media were inoculated at a concentration of 10% (v/v) after being autoclaved at 121 °C for 15 min. Ethanol 1% (v/v) was added to all media and the pH was adjusted to 5.5 with H_2SO_4.

2.3. Pellicles production and purification

Cultures were incubated at 28 °C, 150 rpm, for 7 days. After incubation, the pellicles produced on the surface of each medium were harvested and washed with water and 1% NaOH solution at 80 °C for 1 h and then washed with distilled water to remove the former.

2.4. Dry weight and yield of BC

BC production was recorded as the dry weight of cellulose within the volume of medium in liter ($g\,L^{-1}$). To determine the dry weight of cellulose sheets, the sheets were oven dried at 45 °C for 3 days until reaching constant weight. The pH of the remaining medium was measured after the cellulose sheets were harvested. The yield of biosynthesis process was calculated as follows:

$$\text{Yield}(\%) = \left(\frac{m_0}{C}\right) \times 100 \quad (1)$$

where m_0 is dry weight of cellulose (g) and C is the weight of carbon source (g).

2.5. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of samples was done using a TGA instrument 931 thermal analyzer (TA Instruments). The average weight of samples was approximately 0.6 mg. Scan rates of 20 °C min^{-1} over a temperature range of 20–620 °C were applied. All tests were carried out under inert (N_2) atmosphere.

2.6. X-ray diffraction (XRD)

Dried films of cellulose nanofibers were X-rayed using an X'Pert pro MPD (multi-purpose diffractometer, Model PW3040/60). X-ray diffraction patterns were recorded at the CuKα radiation wavelength (λ = 1.54 Å), generated at a voltage of 40 kV and a filament emission of 30 mA. Samples were scanned from 0–40° 2θ-range at scan speed of 0.5° min^{-1}. The crystallinity (Cr) and crystallite size (CrS) were calculated based on X-ray diffraction measurements. Crystallinity was calculated from the following Eq.:

$$\text{Cr}(\%) = \frac{S_c}{S_t} \times 100 \quad (2)$$

where S_c is sum of net area and S_t is sum of total area. The Cr*S* was determined using Scherrer equation as following:

$$\text{Cr}S = \frac{K\lambda}{\beta \cos\theta} \quad (3)$$

where K is the shape factor (0.9), λ is the x-ray wavelength (1.54 Å), β is the full width at half maximum height (FWHM), and θ is the Bragg's angle.

2.7. Field emission scanning electron microscopy (FE-SEM)

The samples were mounted and gold-coated in preparation for field emission scanning electron microscopy (FE-SEM) imaging. FE-SEM was performed using the field-emission SEM Hitachi SU 8090. FE-SEM experiment was conducted at an accelerated voltage of 5 kV and magnification of 20k.

3. Results and discussion

3.1. BC production and yield

Fig. 1a shows the amounts of BC production from various carbon sources. After 7 days incubation, the production of the BC was confirmed from three media. However, more BC cultured in media Zhou (Z) and Hestrin-Schramm (H) was produced, respectively. Moreover, it was confirmed that *G. xylinus* could not produce BC in the medium consisting of Yamanaka (Y). The nitrogen sources such as yeast extract and pepton were indispensable for the cell