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In situ modifications to bacterial cellulose with the water insoluble polymer poly-3-hydroxybutyrate

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ABSTRACT

Bacterial cellulose is a pure, highly crystalline form of cellulose produced from the bacteria *Gluconacetobacter xylinus* that has become of increasing interest in materials science due to its nanofibrillar structure, ideal for incorporation into other materials as a reinforcing material. The morphology and properties of bacterial cellulose can be altered by including additives not specifically required for growth of the bacteria in liquid media. The bioplastic poly-3-hydroxybutyrate (PHB), along with hydroxypropylmethyl cellulose (HPMC) and Tween 80 were selected and added to the growth media at different concentrations to examine their impact on the resulting cellulose, leading to changes in yield, crystallinity and morphology. The crystallinity index of the nanofibrils was found to vary greatly when using these different methods to calculate it from XRD data, indicating that particular care must be taken when comparing crystallinity results reported in the literature. PHB was able to be incorporated into the bacterial cellulose fibrils during production, increasing the potential for favourable interactions of the bacterial cellulose microfibrils with a neat PHB matrix with the aim of making a fully degradable nanocomposite system.

1. Introduction

Cellulose is the most abundant polymer on earth, and is becoming of increasing interest because of its fibrillar nature and potential as a reinforcing material in composites, being biodegradable, sustainable and renewable. Cellulose has long been produced from plant sources, however bacterial cellulose (BC), produced in high amounts by *Gluconacetobacter xylinus*, is particularly appealing due to its purity and highly crystalline nanostructure. There have recently been several reports on the amount of cellulose produced by *Gluconacetobacter* grown in different media, often by simply substituting the carbon and/or nitrogen components. A wide range of carbon and nitrogen sources have been investigated in this way, as has the inclusion of additional supplements.

The inclusion of additives in the growth media, that is components in the media that are not specifically required for bacterial cell growth, can affect cellulose production in different ways, as the assembly of cellulose is susceptible to chemical and physical influences by the compounds present during synthesis and aggregation (Uhlin, Atalla, & Thompson, 1995), by binding directly to the cellulose during production and interfering with the crystallization, or co-crystallizing with the cellulose. It is also possible that the additive may interfere with the bacterial cells themselves, thereby altering the cellulose production indirectly. Regardless of the method, the yield, structure, morphology and physical properties can all be affected by the presence of an additive in the media, effectively creating *in situ* modifications.

Water soluble polymers have been included in the culture media of cellulose producing bacteria with conflicting results. Some researchers note that the inclusion of such additives simply results in altered cellulose structure (Cheng, Catchmark, & Demirci, 2009; Tokoh, Takabe, Sugiyama, & Fujita, 2002b), whereas others find the creation of composites as the additive is incorporated into the growing cellulose fibrils, leading to *in situ* composites (Hessler & Klemm, 2009; Seifert, Hesse, Kabrelian, & Klemm, 2004). Water soluble polymers carboxymethyl cellulose and methylcellulose have been added to the media with claims that the inclusion of additives such as these directly affects the cellulose, causing decreased crystallinity and crystal size, as well as greater thermal stability and pore size (Cheng et al., 2009). It has also been reported that the additives become incorporated into the cellulose, creating a composite-type material (Seifert et al., 2004). Other polymers such as Tween 80 (Huang, Chen, Lin, Hsu, & Chen, 2010) and hydroxypropylmethyl cellulose (HPMC) (Huang, Chen, Lin, & Chen, 2011) have also been incorporated into the growth media of cellulose-producing bacteria, with differences observed in pore size, degree of polymerization, crystallinity, fibre widths and mechanical strength.

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Similarly, including additives of poly(ethylene oxide) (Brown & Laborie, 2007), poly(vinyl alcohol) (PVA) (Gea, Bilotti, Reynolds, Soykeabkeaw, & Peijs, 2010) and starch (Grande et al., 2009) in the growth media have resulted in these additives being incorporated into the bacterial cellulose resulting in *in situ* composites, however PVA levels were only achieved up to 1.3%. Composites with poly(ethylene oxide) and starch were achieved with much higher levels of the additives, indicating that it may be possible to make nanocomposites with bacterial cellulose from this method. Results from these works showed that the cellulose was well dispersed, and the nanocomposites typically had good mechanical properties.

In this work, we use poly-3-hydroxybutyrate (PHB) as the key material used for modifying the cellulosic nanofibres during the culture stage. Composites have been reported using bacterial cellulose and the water insoluble polymer PHB by an impregnation method. In these cases, the cellulose pellicle was soaked in a solvent containing dissolved PHB and, as the solvent evaporated, the PHB was incorporated into the spaces between the cellulose fibrils cellulose (Barud et al., 2011; Cai & Yang, 2011; Cai, Yang, & Kim, 2011). While water soluble polymers have been well documented as additives in the culture media for cellulose producing bacteria, the effects of water insoluble polymers in the media is unknown. However in this work, a non water soluble polymer, PHB, was directly dispersed in bacterial cellulose culture medium. HPMC and Tween 80 were selected as water soluble polymers that have previously been investigated in the media for a variety of cellulose-producing bacteria, and were examined for comparison. Alterations in the structure of bacterial cellulose may be desirable for the creation of composites in that if the fibrils become more "PHB-like", they may improve interaction if incorporated into a PHB matrix to form a reinforced, fully degradable nanocomposite.

2. Experimental

2.1. Bacterial strain

A culture of cellulose-producing *G. xylinus* ATCC 53524 was kindly provided by Gary Dykes from the School of Science, Monash University, Malaysia.

2.2. Media

The media used to cultivate *G. xylinus* was Hestrin–Schramm (HS) (Schramm & Hestrin, 1954), with different concentrations (described below) of additives added. Media were adjusted to pH 5.0 with HCl or NaOH and autoclaved at 121 °C for 20 min. The additives used were HPMC, Tween 80 and PHB. HPMC was obtained from Dow Chemical, and Tween 80 and PHB were obtained from Sigma–Aldrich.

2.3. Growth conditions

Seed cultures were prepared by selecting a single colony from a working plate of Hestrin–Schramm agar and inoculating 10 mL of HS broth. These cultures were incubated for seven days at 28 °C under static conditions. Following growth, seed cultures were shaken vigorously to remove the bacterial cells from the cellulose pellicle. Pellicles were removed and the resulting cell suspension was used for inoculations. Cultures were grown in 200 mL conical flasks containing 50 mL of media and were inoculated at a concentration of 1% of the cell suspension. Cultures were incubated for seven days at 28 °C under static conditions. All cultures were grown in triplicate. Additional pellicles were produced in HS media containing 1 wt% PHB for tensile tests.

2.4. Treatment of cellulose films

Following incubation periods, cultures were shaken vigorously to remove the attached bacterial cells. Pellicle films were removed from cultures and rinsed to remove any residual media. Pellicles were washed with 0.1 M NaOH at 80 °C for 1 h, and then washed repeatedly until a neutral pH was obtained and dried at room temperature. Pellicle films were weighed once dry.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed using the field-emission SEM JEOL 7001F. Samples were coated with a gold/palladium coating, and were examined at 5 kV.

2.6. Fourier-transform infra-red

Fourier transform-infra red (FTIR) spectroscopy was completed using Perkin-Elmer Spectrum 100 Spectrometer. Scans were completed between 4000 and 450 cm^{-1} with 16 convolutions. Baselines for each sample spectrum were normalized using the Spectrum software. I_α content was calculated using the peak heights at 750 and 710 cm^{-1} by the equation determined by Yamamoto, Horii, and Hirai (1996). In addition, cellulose pellicles from HS media and HS media containing 1 wt% PHB were ground into a fine powder and mixed with potassium bromide (KBr) powder, dried under vacuum and pressed into small discs for examination by FTIR according to the protocol described above. Neat PHB powder was also examined in this way.

2.7. X-ray diffractometry

X-ray diffraction (XRD) was used to monitor the d_{1-10} spacing corresponding to the interlayer spacing of the crystalline structure of the bacterial celluloses. The XRD measurements were performed on the cellulose sheet samples using a Bruker D8 Diffractometer operating at 40 kV, 40 mA, Cu Kα radiation monochromatised with a graphite sample monochromator with a diffractogram recorded between 2θ angles of 2° and 40°. Crystallite size was calculated using the software TOPAS™. The FWHM (full width at half maximum height) for the two major peaks was used for this calculation, as the third peak could not provide reliable FWHM values due to its low intensity. Calculations were conducted using the Scherrer equation with a shape factor constant of 1, and an instrument FWHM of 0.068° 2θ. Crystallinity was also calculated using TOPAS™ based on the method of Hindeleh and Johnson (1971). The amorphous area was determined using International Centre for Diffraction Data (ICDD) PDF card 00-060-1501, amorphous cellulose. The crystalline peak positions were selected based on positions given in Czaja, Romanovicz, and Brown (2004). A pseudo Voigt Function was used to profile the peak shape and area for both the amorphous and crystalline components.

2.8. Solvent casting PHB films

A neat PHB film was prepared by dissolving 5 wt% PHB in chloroform under mechanical stirring at 80 °C for 3 h. The films were cast in glass petri dishes and the solvent was allowed to evaporate at room temperature. These films were examined for tensile properties for comparative purposes only.

2.9. Tensile properties

Tensile strength, elongation at break and modulus were determined for cellulose produced in standard HS media and media