Carbohydrate Polymers 92 (2013) 1717–1723

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

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

Dianne R. Ruka[a,b], George P. Simon[b], Katherine M. Dean[a,*]

[a] *CSIRO Materials Science & Engineering, Gate 5 Normanby Road, Clayton 3168, Australia*[1]
[b] *Department of Materials Engineering, Monash University, Clayton 3800, Australia*

ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 7 September 2012
Accepted 1 November 2012
Available online 9 November 2012

Keywords:
Bacterial cellulose
In situ
Modifications
Poly-3-hydroxybutyrate
Additives
Crystallinity

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.

* Corresponding author. Tel.: +61 3 9545 2686; fax: +61 3 9544 1128.
E-mail addresses: dianne.ruka@csiro.au (D.R. Ruka), george.simon@monash.edu (G.P. Simon), katherine.dean@csiro.au (K.M. Dean).
[1] http://www.csiro.au.

0144-8617/$ – see front matter.
http://dx.doi.org/10.1016/j.carbpol.2012.11.007

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

containing 1 wt% PHB, and a solvent cast PHB film on an Instron universal testing machine (model 3366) and tested in accordance with ASTM D882 (using a type IV specimen as described in ASTM D638). The Instron was fitted with a 100 N static load cell, pneumatic grips, and the speed of extension was set to 2 mm/min. A minimum of ten specimens per each formulation were tested until fracture, from which a mean and standard deviation were calculated.

3. Results and discussion

3.1. Film weight

Each additive was added to the culture at four different concentrations (Table 1). The weight of the film from each culture was examined. The percentage increase in weight of each film from the cellulose produced in the absence of the additive is shown in Fig. 1.

Various additives have been reported to interfere with the production of cellulose by interfering with aggregation of microfibrils during production (Benziman, Haigler, Brown, White, & Cooper, 1980), which can result in decreased cellulose yield. An increase in the weight of the cellulose pellicle can indicate an increase in cellulose production, likely due to an increase in cell growth rate, or an increase in weight may be the result of the incorporation of the additive into the pellicle film. Differences observed in the structure and morphology, specifically in the fibril appearance and width, and crystallite sizes and crystallinity, are discussed below. However, even though an additive may provide beneficial characteristics for bacterial cellulose, and allow tailored design of the cellulose for specific purposes, if an additive results in significant decreases in yield of cellulose, the cost of production of the cellulose would increase, making the production undesirable for large-scale operations. Consideration should thus be given to all factors, including yield, when seeking to obtain specific characteristics in bacterial cellulose.

The inclusion of HPMC as an additive in the media decreased the weight of the film at low concentrations, but increased the weight at higher concentrations, though no difference was observed between the weight at high concentrations of HPMC and the control. It is believed that the increase observed was not due to a stimulation in the cell growth rate, but rather an indication that HPMC was incorporated into the pellicle film, thus causing the increase in weight at higher concentrations. The opposite is true, however, for Tween 80 that caused a fairly consistent decrease in the weight of the film, indicating that this additive negatively impacts cellulose production. These two additives were selected for use as comparison to PHB as they have previously been shown to have an affect on the structure of bacterial cellulose. HPMC is a water-soluble polysaccharide that can be used as an emulsifier, whereas Tween 80 is a water soluble polyethylene sorbitol ester that has a range of uses such as solubilizing proteins.

The inclusion of PHB, ranging from 0.25 to 1.0 wt% PHB in the media, resulted in a significant increase in weight of the pellicle film produced. PHB was present on the surface of the pellicle though attempts were made to remove the PHB powder from the pellicle surface during the washing steps. Based on the increase in film weight, it is likely that the product formed is a BC–PHB material. Further testing of this material by FTIR, SEM and tensile testing was completed to confirm the presence of PHB in the film.

Table 1
Concentrations added to HS media for each additive.

Additive	Concentration point (wt%)			
	1	2	3	4
Hydroxypropylmethyl cellulose	0.25	0.5	1.0	2.0
Tween 80	0.05	0.1	0.2	0.4
Poly-3-hydroxybutyrate	0.125	0.25	0.5	1.0

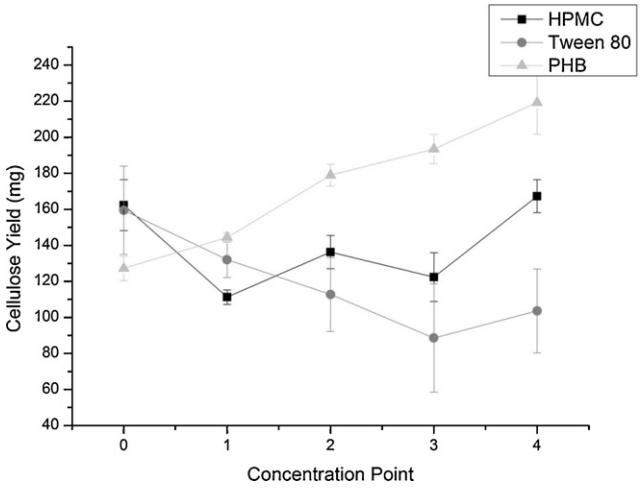

Fig. 1. Cellulose yields obtained from cultures with different concentrations of additives in the media.

3.2. Bacterial cellulose morphology and crystal structure

Bacterial cellulose is produced in the cell's cytoplasmic membrane, and is extruded as microfibrils of approximately 1.5 nm in width, and the microfibrils aggregate into a ribbon-shaped fibril approximately 40 nm in width (Ross, Mayer, & Benziman, 1991). Various additives included in the media can act as co-polymers becoming incorporated into the bacterial cellulose as it is produced, or can bind to the cellulose, affecting the morphology and structure of the cellulose (Huang et al., 2010; Klemm et al., 2006; Tokoh, Takabe, Sugiyama, & Fujita, 2002a; Yamamoto et al., 1996). By contrast, other additives, antibiotics for example, directly affect the bacterial cell and therefore the production of the cellulose as a result (Yamanaka, Ishihara, & Sugiyama, 2000).

In the work described below, one concentration of each additive was selected and the morphology of the film was examined. This involved examination by SEM for fibril morphology and width, by XRD for crystallite size and crystallinity and also by FTIR for its crystalline cellulose I_α content.

When viewed by SEM, bacterial cellulose typically presented as an interwoven mesh of fibrils of approximately 40 nm in width, although the widths of fibrils were subject to variance due to their biological nature. The inclusion of some additives resulted in some changes to the morphology of the cellulose (Fig. 2). The addition of HPMC resulted in slightly thinner fibrils, however the difference was not statistically significant, the HPMC fibrils also appeared straighter (Fig. 2b). Tween 80 as an additive resulted in slightly wider fibrils at 56 nm, over the 40 nm fibrils present without an additive (Table 2). It is possible that this additive impacted bacterial synthesis, since Tween 80 has been shown to stimulate glucan production, a glucose polysaccharide, in *Streptococcus mutans*, however no effect on bacterial cells was observed (Umesaki, Kawai, & Mutai, 1977), whereas it has been shown to decrease mechanical strength of bacterial cellulose (Huang et al., 2010).

PHB is of particular interest, its incorporation in the growth medium of bacterial cellulose not having been reported previously. PHB is water insoluble and is produced intracellularly by particular bacterial species, such as *Azotobacter*, *Bacillus* and *Pseudomonas* species (Byrom, 1987). PHB was dispersed in the media used to produce bacterial cellulose. Based on the weight of the pellicles as described above, as well as the SEM of this cellulose, it appears that PHB was integrated into the cellulose during the synthetic

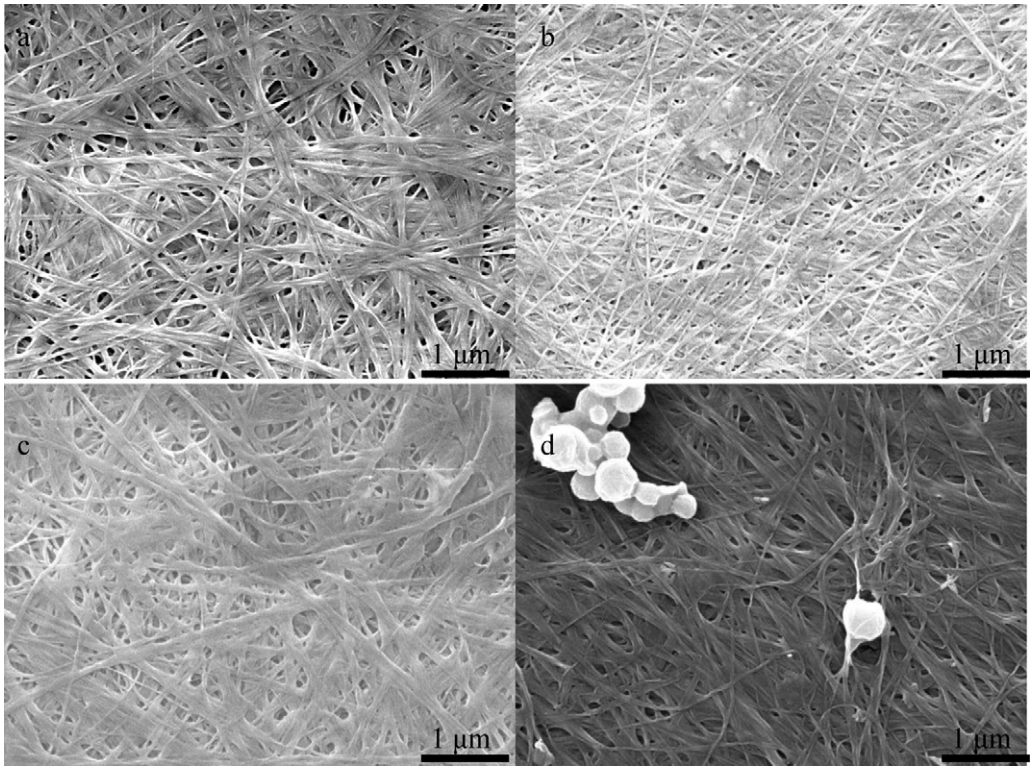

Fig. 2. SEM images of cellulose produced in media with no additives (a), HPMC (b), Tween 80 (c) and PHB (d).

process. PHB can be seen on the surface of the cellulose fibrils in Fig. 2d and was examined in further detail in Fig. 3. Fig. 3 shows the appearance of PHB powder before its inclusion in the media, and the similar appearance on the cellulose. In addition to the PHB on the surface of the pellicle (Fig 3b), PHB was also observed to be interwoven amongst the cellulose fibrils on the underside of the pellicle (Fig. 3c and d). There have been several papers published involving the inclusion of water soluble polymers in the media for cellulose producing bacteria (Chao, Mitarai, Sugano, & Shoda, 2001; Hessler & Klemm, 2009; Seifert et al., 2004; Tokoh et al., 2002a; Yamamoto et al., 1996). Some have been reported as altering the structure of the cellulose, whereas others were actually incorporated into the cellulose fibrils during synthesis. To the best of our knowledge, insoluble polymers have not been examined. Since bacterial cellulose is formed as a pellicle on the surface of the media, if an insoluble polymer is present at the air/surface interface, it is likely that it too can be incorporated into the mesh of cellulose fibrils. This was not the case with PHB as it accumulated at the bottom of the flask. It may be that some PHB remained dispersed in the medium, or that the bacterial cells were able to access the PHB from the bottom of the flask as it appeared that the PHB at the bottom of the flask become attached to the pellicle. Regardless, the PHB was incorporated in amongst the cellulose fibrils, indicating that this may represent a more general pathway for insoluble polymers be incorporated, and may provide bacterial cellulose with improved *in situ* modifications.

From Fig. 3, it is difficult to tell exactly how much PHB was incorporated into the cellulose. From the top view of the pellicle (Fig. 3b), it appears as though the PHB has largely coated the surface, as the fibrils are packed too tightly to observe any incorporated PHB, however from the bottom of the film (Fig. 3c and d), it is possible to visualize the mesh of cellulose fibrils and PHB.

As previously described, additional substrates acting as a host polymer have been added to the media used to produce bacterial cellulose in order to produce *in situ* composites with starch (Grande et al., 2009) and poly(ethylene oxide) (Brown & Laborie, 2007), the amount of matrix in those composites was not achieved with PHB, as most of the pellicle consisted of cellulose. We hypothesize that the cellulose produced in the presence of PHB will have a higher affinity to this material over cellulose produced in traditional media, and could be used as reinforcement material in a PHB matrix.

Cellulose I is the form of cellulose found in nature, it is composed of parallel chains (Delmer, 1987) and exists in two distinct allomorphs, I_α and I_β (Atalla & Vanderhart, 1984). The ratio of cellulose I_α and I_β produced in nature depends on the organism producing it. Changing the media composition has been shown to affect the amount of cellulose I_α produced by *G. xylinus* (Klemm et al., 2006).

Table 2
Structural values obtained from bacterial cellulose produced in the presence of additives.

Additive	Concentration	Fibril width (nm)	Cellulose I_α content (%)	Crystallinity (%) (calculated from one amorphous peak)	Crystallinity (%) (calculated from four amorphous peaks)	Crystallite size (nm)
No additive	–	40	68	86	79	6.9
Hydroxypropylmethyl cellulose	1%	38	70	65	60	5.8
Tween 80	0.1%	56	68	87	35	6.8
Poly-3-hydroxybutyrate	0.5%	46	69	69	52	6.8

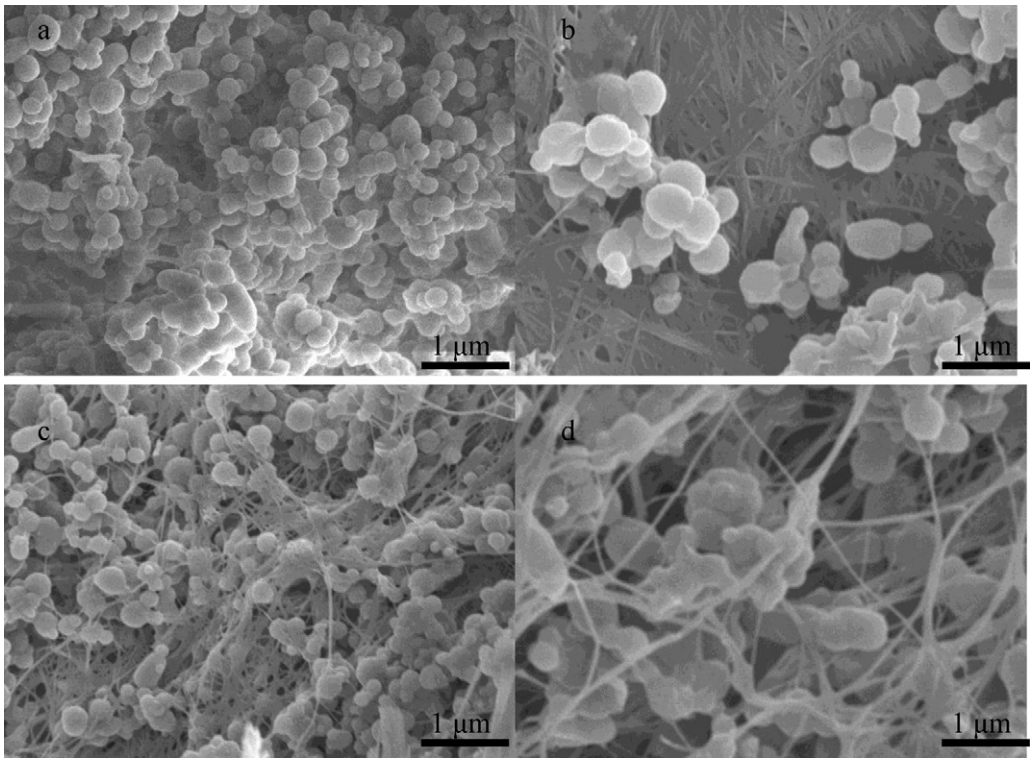

Fig. 3. SEM images of PHB powder (a) and bacterial cellulose grown with PHB in the media (b–d).

The amount of cellulose I_α can be calculated from FTIR peaks at 750 and 710 cm^{-1} (Fig. 4a). The additives included here showed no impact on I_α content, all ranging from 68 to 70% (Table 2). These results all remain very high, indicating that these additives certainly do not have a significant negative effect on the ratio of I_α/I_β content, and that the bacterial cellulose remains high in I_α content.

From the FTIR of the two pellicle films obtained from standard HS media and HS media with PHB as an additive (Fig. 4a), an additional peak was seen in the BC-PHB film at approximately 1724 cm^{-1}. This was confirmed by grinding up cellulose and cellulose grown in the presence of PHB (BC–PHB) and made into KBr discs. PHB was also examined in this way and can be seen in Fig. 4b. The peak at 1724 cm^{-1} which is seen both in the PHB and BC–PHB curves, but not in the BC curve, can be thus attributed to the C=O group which is present only in PHB, supporting the hypothesis that the pellicle produced in the presence of PHB is not simply cellulose alone, but a combination of bacterial cellulose and PHB.

If we assume that the increase in cellulose weight is due entirely to the addition of PHB then we could predict that with 1 wt% PHB in the media, we obtain a combined BC–PHB pellicle of approximately 40 wt% PHB. However it is likely that much of this PHB is superficially attached to the surface rather than being integrated amongst the fibrils. From the general appearance of the pellicle, it would not appear that it consists of such a large amount of PHB. It is also possible that PHB was used as a carbon source for the bacterial cells and led to the increased production of cellulose this way, as PHB is itself naturally an intracellular storage molecule that can be broken down by bacterial cells for cell metabolism. Based on the presence of an additional peak in the BC-PHB material at approximately 1724 cm^{-1} due to the carbonyl group in PHB, we can conclude that the pellicle film must contain some PHB.

Bacterial cellulose exists as a highly crystalline material with small crystallite sizes and XRD data was used to look at the size aspect of these crystallites from the XRD peaks (Fig. 5).

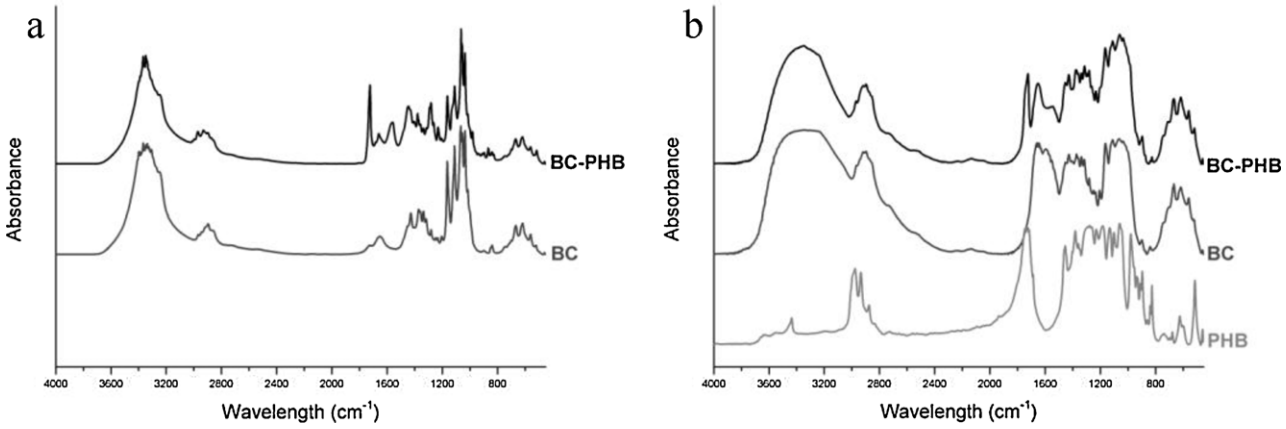

Fig. 4. FTIR obtained from BC grown in media with and without PHB as a film (a) and from KBr discs (b).

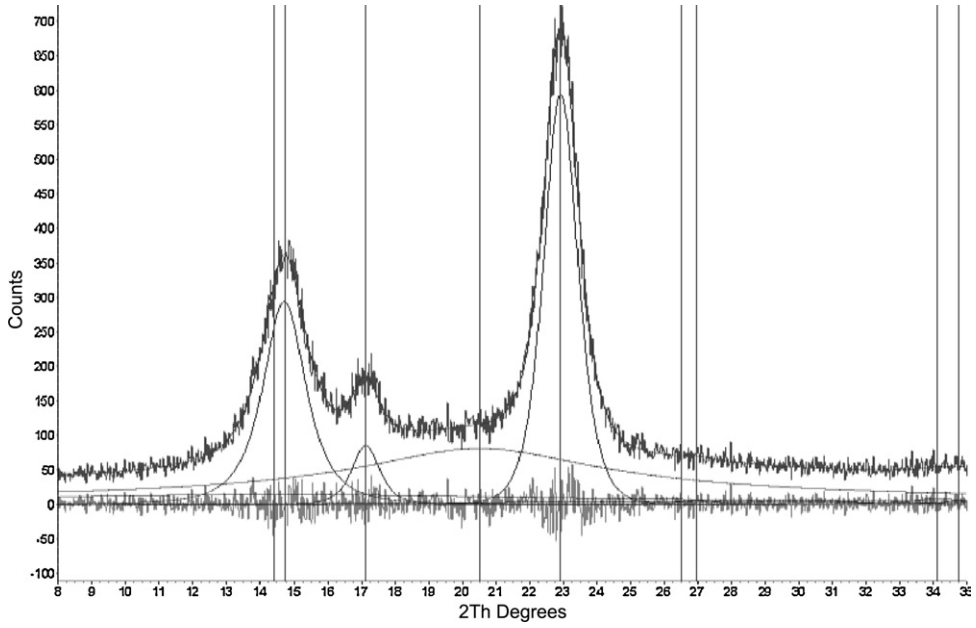

Fig. 5. XRD pattern of bacterial cellulose with peaks for the calculation of crystallite size and crystallinity.

The degree of crystallinity can be calculated using XRD data comparing a single defined amorphous peak to the crystalline peaks obtained from cellulose produced here, with the crystallinity values also typically very high. However, when these results were repeated using four peaks to define the amorphous area, the crystallinity values were very different (Table 2). Increasing the amorphous peaks from one to four caused a decrease in the calculated value for crystallinity for the cellulose produced in the absence of any additives, however this decrease was very small, indicating that bacterial cellulose is highly crystalline and that these results are generally quite robust to analysis techniques.

The inclusion of the additives caused a decrease in the crystallinity for the cellulose as demonstrated by the calculations completed using four amorphous peaks. HPMC and PHB only decreased the crystallinity to 60 and 52%, respectively, but Tween 80 caused a large decrease in the crystallinity, reducing it to 35%. This is further evidence that these additives all have an impact on the production of the cellulose. Similar to bacterial cellulose, PHB is a semi-crystalline material, however it appears that its incorporation into the cellulose pellicle interferes with the cellulose crystallization. This is not unusual as it is known that polymerization and crystallization are coupled processes in bacterial cellulose production (Benziman et al., 1980). The inclusion of additives in the media can interfere with these processes, leading to changes in the bacterial cellulose produced. Based on observations made in this work, it appears that the inclusion of many additives to the media used to produce bacterial cellulose causes undesirable changes to the cellulose.

Here, we used four amorphous peaks based on the cellulose diffraction pattern provided by the ICDD, however there are different methods that can be used to determine crystallinity (Segal, Creely, Martin, & Conrad, 1959). Based on the differences we observed between the crystallinity data calculated with one amorphous peak and four amorphous peaks, extreme caution should be taken not to over interpret the data presented in literature.

Crystallite sizes remained small, even the presence of additives, ranging from 5.8 to 7.0 nm, however these differences were not statistically significant. The addition of HPMC resulted in smaller crystallite sizes being produced of dimension 5.8 nm, further demonstrating its interference in the polymerization and crystallization process.

3.3. Tensile properties

Preliminary investigations of tensile strength in bacterial cellulose films grown in HS media with and without PHB as an additive were completed, as well as a solvent cast PHB film for comparison, in order to provide further evidence of the incorporation of PHB into the BC film. Sections were cut from the dried pellicle films and examined for the tensile properties. A decrease in tensile strength and modulus was observed from the BC to the BC–PHB film, however the elongation at yield appeared a little higher (albeit with questionable statistical significance), however the BC–PHB film exhibited better mechanical properties across all three parameters as compared to the neat PHB (Table 3).

The BC and BC–PHB films exhibited similar values for stress and strain at break (Fig. 6), however both the BC and BC–PHB films exhibited much better properties than the PHB alone.

These results are similar to mechanical properties achieved by others in the literature. Barud et al. (2011) and Cai et al. (2011) both soaked a BC pellicle in a solvent with dissolved PHB and allowed the solvent to evaporate so the PHB would be incorporated into the BC fibrils. Barud et al. (2011) reported an increase in both tensile strength and Young's modulus in a BC–PHB composite over the BC alone, but only at a low concentration of PHB. As the PHB content increased, the mechanical properties decreased. Cai et al. (2011) however reported an increase in tensile strength from the BC film to the 50:50 BC–PHB composite, but a decrease in modulus.

As there have been changes in the mechanical properties from the BC to the BC–PHB film, this further supports that the inclusion of PHB in the media results in the incorporation of this water insoluble polymer into the BC film. If the cellulose produced with this or other additives is to be considered further as a reinforcing agent, then other properties should also be considered. However based on the typical decrease in both cellulose weight and crystallinity upon

Table 3
Tensile properties of BC, PHB and BC–PHB films.

Film	Tensile strength (MPa)	Elongation at yield (%)	Modulus (MPa)
BC	105.66 ± 9.44	6.57 ± 1.73	1866 ± 451
PHB	21.30 ± 4.24	3.64 ± 0.91	852 ± 171
BC–PHB	67.41 ± 18.22	7.74 ± 1.97	1098 ± 105

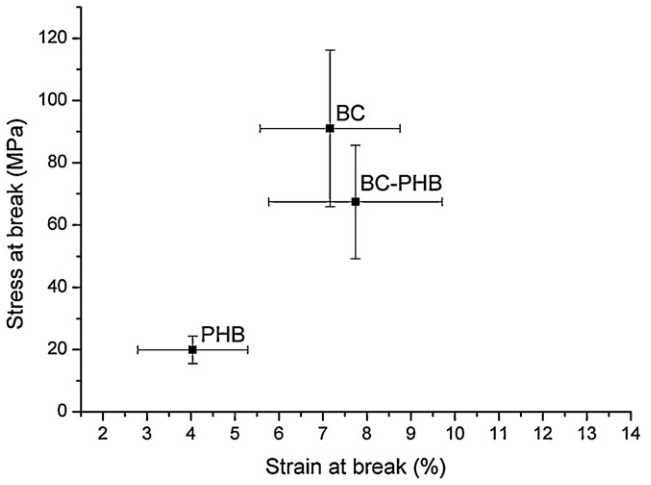

Fig. 6. Stress/strain values for BC, PHB and BC–PHB films.

inclusion of additives, and the observed decrease in tensile strength between neat cellulose and cellulose grown with PHB as an additive in the media here, a high cellulose-producing media without additional components may end up being most appropriate as an additive in nanocomposites. It is also apparent that water insoluble polymers can affect the cellulose as it is produced, creating *in situ* modifications.

4. Conclusions

Incorporation of additives not specifically required for the growth of *G. xylinus* cells or the production of bacterial cellulose can alter the yield, structure and morphology of the cellulose produced. The inclusion of PHB in the media appears to fortuitously result in a composite BC–PHB material. Such compatibilised structures may be a source of reinforcement particularly suited for incorporation in a composite, where the matrix is itself PHB.

Acknowledgments

We thank the Monash Centre for Electron Microscopy for the use of their electron microscopes, and Liz Goodall of CSIRO for the experimental XRD work and calculations. This work was funded by a Julius Career Award from the CSIRO Office of the Chief Executive.

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