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Laccase-assisted grafting of poly(3-hydroxybutyrate) onto the bacterial cellulose as backbone polymer: Development and characterisation

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ABSTRACT

Bacterial cellulose (BC) exhibits high purity, mechanical strength and an ultra-fine fibrous 3-D network structure with bio-compatible and bio-degradable characteristics, while P(3HB) are a bio-degradable matrix material derived from natural resources. Herein, we report a mild and eco-friendly fabrication of indigenously isolated P(3HB) based novel composites consisting of BC (a straight-chain polysaccharide) as a backbone polymer and laccase was used as a grafting tool. The resulting composites were characterised by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), differential scanning calorimetry (DSC), dynamic mechanical analyser (DMA) and water contact angle analyser (WCA). The FTIR spectra of the pure P(3HB) and P(3HB) containing graft composites [P(3HB)-g-BC] showed their strong characteristic bands at 3358 cm^{-1}, 1721 cm^{-1} and 1651 cm^{-1}, respectively. A homogenous dispersion of P(3HB) in the backbone polymer of BC was achieved as evident by the SEM micrographs. XRD pattern for P(3HB) showed distinct peaks at 2θ values that represent the crystalline nature of P(3HB). While, in comparison with those of neat P(3HB), the degree of crystallinity for P(3HB)-g-BC decreased and this reduction is mainly because of the new cross-linking of P(3HB) within the backbone polymer that changes the morphology and destroys the crystallites. Laccase-assisted graft composite prepared from P(3HB) and BC was fairly flexible and strong, judged by the tensile strength (64.5 MPa), elongations at break (15.7%), and Young's modulus (0.98 GPa) because inherently high strength of BC allowed the mechanical properties of P(3HB) to improve in the P(3HB)-g-BC composite. The hydrophilic property of the P(3HB)-g-BC was much better than that of the individual counterparts which is also a desired characteristic to enhance the biocompatibility of the materials for proper cell adhesion and proliferation.

1. Introduction

Since the last few decades, there has been a continuously increasing interest for stronger, stiffer, lighter-weight, and multifunctional engineering material development for potential applications. Many of the polymers and fibres used in materials are being generated mainly from non-degradable petroleum-based polymeric resins, such as epoxies and polyurethane, and high-strength fibres, such as graphite, or aramids. The environmental impact of persistent plastic-based wastes is increasing global concerns and disposal methods are limited. In addition, the petroleum resources are finite and becoming increasingly costly. Therefore, in this contest, recently bio-based composite materials or blends are being engineered for target applications in different industries to address the growing environmental concerns of a globally unsustainable dependence on petroleum-based resources (Srubar et al., 2012; Iqbal, Kyazze, & Keshavarz, 2013; Yadav, Rhee, & Park, 2014). Bio-polymers generated from renewable natural sources by means of micro-organisms are often biodegradable/biocompatible and non-toxic. Among the most promising and well-characterise bio-polymers, P(3HB) are of particular interest to prepare composites. P(3HB) graft composites with cellulose such

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as P(3HB)-g-cellulose, which is the focus of the work presented here, to improve or impart new physio-chemical and thermal properties, decrease brittleness, and increase tensile strength of P(3HB) with increased cellulosic contents. One possible explanation for the enhanced flexibility of cellulose is its overall hydrogen bonding density that is responsible to decrease the brittleness and improve the tensile strength of P(3HB) based composites. Depending on the physio-chemical nature and compatibility of the individual constituent surfaces, either P(3HB) or cellulose, various interactions can occur at the interface. Interfacial chemical reactions can lead to various intermolecular bonds such as hydrogen bonding type interactions. Surface functionalities of P(3HB) and cellulose include moderate polar ()C=O) and polar groups (—OH and —COOH) and one primary and two secondary hydroxyl groups, respectively which possibly interact (Singh & Mohanty, 2007), and generate some new hydrogen bonds during composite formation.

Grafting plays an important role in the modification of polymers to improve or impart ideal/new properties that individual materials fail to demonstrate on their own (Srubar et al., 2012). There are several means to modify polymers to make them useful for a wider range of applications (Akaraonye, Keshavarz, & Roy, 2010). These techniques include alkaline hydrolysis, chemical, gamma radiation, photochemical, UV, plasma-induced and enzymatic grafting (Kim, Chung, Kim, Kim, & Rhee, 2002; Lao, Renard, Linossier, Langlois, & Rehel, 2007). Enzymatic grafting of bio-polymers is quite a new and green approach and the grafted materials can find potential applications in various fields. The principle involved is that enzymes offer clean and safe alternatives to current practices as an active starting material in the grafting reaction and safe conditions (Aljawish et al., 2012). There are several potential advantages for the use of enzymes in polymer synthesis and modifications with respect to health and safety, enzymes offer the potential of eliminating the hazards associated with reactive reagents. A potential environmental benefit for using enzymes is that their selectivity may be exploited to eliminate the need for waste full protection and de-protection steps.

Apart from plants, cellulose is also produced by bacterial genera *e.g. Rhizobium* spp. *Agrobacterium* spp. *Acetobacter* spp. *Alcaligenes* spp. Bacterial cellulose (BC) is a straight-chain polysaccharide with the same chemical structure as cellulose that is derived from plants. However, bacterial cellulose has the advantage of being devoid of lignin, pectin, hemicellulose and other biogenic products that are normally associated with plant cell walls (Jonas & Farah, 1998). Due to the high purity and special physicochemical characteristics, bacterial cellulose has applications in a wide range of areas including food, bio-medical and tissue engineering (Klemm, Schumann, Udhardt, & Marsch, 2001; Silva et al., 2014). However, the potential for bacterial cellulose in reinforcing polymers is not widely known. Therefore and in light of the above-discussed characteristics, bacterial cellulose would appear to be an interesting potential candidate for the development of high-strength bio-composites.

In the present work, novel graft composites combining the advantages of BC backbone and P(3HB) were prepared using laccase as a grafting tool. Enzyme-based biotransformation of biopolymers with natural origin *i.e.*, BC (a straight-chain polysaccharide) and P(3HB) into the value-added graft material presents a unique opportunity for the production of "green" composites. Keeping in view the increasing environmental consciousness and demands of legislative authorities, and the fact that the potential of green composites in different sectors of the modern biotechnological world needs to be extensively explored, the present study was carried out to synthesise and characterise a P(3HB) and BC- based novel composite to present its potential for various applications.

2. Materials and methods

2.1. Microbial cultures

In the present study two bacterial cultures *i.e.*, *Acetobector xylinus* (strain-ATCC 700178) and *Bacillus subtilis* (strain-NCTC 3610) were used for the production of bacterial cellulose and P(3HB), respectively. Both of the strains ATCC 700178 and NCTC 3610 were obtained from the culture collection unit of the University of Westminster, London, UK.

2.2. Maintenance of B. subtilis

The Gram-positive *B. subtilis* was grown in a temperature controlled incubator at 30 °C for 24 h in a sterile nutrient broth for the development of a homogenous inoculum suspension. The main constituents of the nutrient broth were as follows: 1.0 g/L; yeast extract, 2.0 g/L; peptone, 5.0 g/L; sodium chloride, 5.0 g/L. After the stipulated time period (24 h), the incubated bacterial culture was used subsequently for the production of P(3HB) using a modified G medium (MGM).

2.3. Maintenance of A. xylinus

A pure culture of bacterial strain ATCC 700178 was maintained at 4 °C in nutrient liquid medium after sterilisation at 121 °C and 103 kPa for 10 min. The main constituents of nutrient liquid medium were 4% (w/v) glucose, 0.1% (w/v) yeast extract, 0.7% (w/v) polypeptone, 0.8% (w/v) $Na_2HPO_4{\cdot}12H_2O$. A sub culture of *A. xylinus* (strain-ATCC 700178) was used for the production of bacterial cellulose.

2.4. Production and extraction of P(3HB)

P(3HB) production was carried out in 500 mL Erlenmeyer flasks with a 150 mL working volume using a modified G medium (MGM), (the main constituents of the MGM were (g/L): $FeSO_4$, 0.005; $(NH_4)_2SO_4$, 2.00; $MnSO_4{\cdot}H_2O$, 0.003; $MgSO_4{\cdot}7H_2O$, 0.41; K_2HPO_4, 0.50; $CaCl_2{\cdot}2H_2O$, 0.10; $CuSO_4{\cdot}5H_2O$, 0.0005; $ZnSO_4{\cdot}7H_2O$, 0.0005; $CoCl_2$, 0.005; Yeast extract, 2.50 and sucrose, 20.0) (Akaraonye et al., 2010). A freshly prepared inoculum (as discussed in Section 2.2) was used to ferment the MGM to produce P(3HB). Sucrose was sterilised separately before adding into each of the flasks along with the inoculum. After 72 h fermentation P(3HB) was extracted from the cells using the chloroform–hypochlorite dispersion method (Rai et al., 2011).

2.5. Production and extraction of BC

A. xylinus (strain-ATCC 700178) was grown in medium containing 4% (w/v) glucose, 0.1% (w/v) yeast extract, 0.7% (w/v) polypeptone, and 0.8% (w/v) $Na_2HPO_4{\cdot}12H_2O$ in addition with 1.4% (v/v) ethanol. The pH of medium was adjusted to 5.5 before inoculation. Batch cultures in flasks were incubated at 26 °C for 7 days under continuous shaking at 130 rpm. Hydrated BC pellicles were harvested from 7 days old fermented culture, washed three times with sterilised water and kept immersed overnight in sterilised water to remove excess chemicals from the BC.

2.6. Synthesis of graft composite

A fungal laccase from *Trametes versicolor* was used as a grafting tool which was obtained from Sigma–Aldrich, USA. Freshly extracted P(3HB), and BC were treated with laccase using sodium malonate buffer of pH 4.0 at room temperature and stirred continuously. The above treated mixture was then poured into the sterile

labelled Petri plate followed by the incubation in hot air oven at 50 °C. After stipulated incubation time a fine and smooth membrane was obtained. The resulting enzymatically grafted membrane was designated as P(3HB)-g-BC. Three different vaporisation rates (fastest, medium and lower) were trialled for preparing the composite samples. The fastest rate involved placing the samples under a vacuum during drying. A medium vaporisation rate was achieved by drying the samples in hot air oven at 80 °C. While, a lower vaporisation rate was achieved by placing the sample in a covered Petri dish with only a small gap between the edge of the dish in hot air oven at 50 °C. With the fastest vaporisation rate, the composites produced were severely shrivelled after desiccation. At the medium rate, the grafted composites contained some visible air bubbles inside the sample. At the lowest vaporisation rate, the composite membranes did not have any trapped bubbles therefore the lowest vaporisation rate was followed throughout the further preparation of graft composites synthesis for characterisation studies.

2.7. Materials characterisation

2.7.1. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was used primarily to identify the chemical structure of the P(3HB), BC, P(3HB)-g-BC and P(3HB)-g-BC* composites (*: prepared in the absence of laccase). Each of the individual polymer and grafted composites were placed directly onto the diamond crystal, and infrared absorption spectra were recorded from the wavelength region of 4000–500 cm^{-1} using a Perkin-Elmer System 2000 FT-IR spectrophotometer.

2.7.2. Scanning electron microscopy (SEM)

Surface morphologies and microstructure of the individual polymers *i.e.*, P(3HB), and BC and grafted composites *i.e.*, P(3HB)-g-BC and P(3HB)-g-BC* were studied in ultra-high vacuum mode at an accelerating voltage of 5 kV in a field emission gun scanning electron microscope (Philips, XL-30, FEG SEM; EFI, Netherlands). The samples were placed on the 8 mm diameter aluminium stubs and gold coated for 2 min using the gold spluttering device (EMITECH-K550). High definition images (HDI) were then recorded at different magnifications to study the surface morphologies of each sample.

2.7.3. Differential scanning calorimetry (DSC)

In the present work a Pyris Diamond Differential scanning calorimeter (Perkin-Elmer Instruments, USA) was used to measure the thermal properties of the individual polymers and their grafted composites using DSC ~3–7 mg of sample weight. Samples were encapsulated in standard aluminium pans to avoid contamination of the ampoules. The temperature scanning range was from −57 to 250 °C and the scanning rates were 20 °C/min under nitrogen atmosphere.

2.7.4. Dynamic mechanical analyser (DMA)

Dynamic mechanical analyser measurements *i.e.*, Young's modulus, tensile strength and elongation at break of the individual polymers and grafted composite were carried out on a Perkin-Elmer Dynamic Mechanical Analyser (DMA) Q800. The load was set within a range of 1–6000 mN and the crosshead speed was set at a constant tensile rate of 200 mN min^{-1}.

2.7.5. X-ray diffraction (XRD)

The crystalline structures of the individual and grafted composites were examined by wide-angle X-ray diffraction with a thin film attachment using a Brüker D-8 Advance X-ray diffractometer equipped with Ni filtered Cu K radiation. The samples were scanned at 2θ values from 10 °C to 100 °C with a scan speed of 5 °C/min.

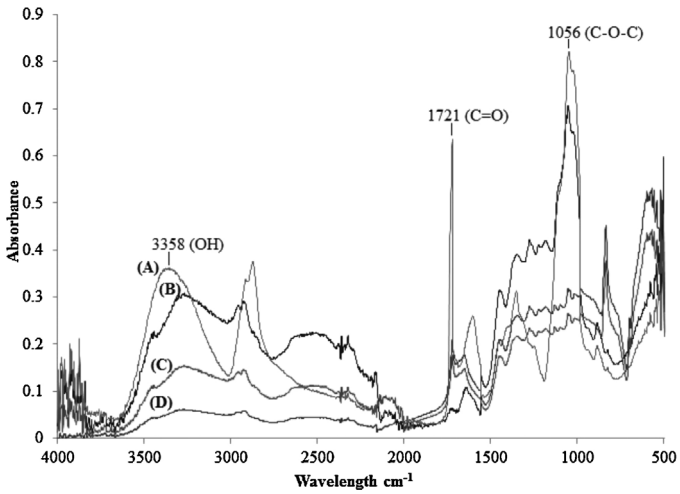

Fig. 1. Typical spectra of the individual polymers and their graft composites taken from FT-IR; top to bottom (A) P(3HB)-g-BC; (B) BC; (C) P(3HB)-g-BC* and (D) P(3HB).

2.7.6. Water contact angle (WCA)

The water contact angles of the P(3HB), BC, and the resulting copolymer *i.e.*, P(3HB)-g-BC and P(3HB)-g-BC* were measured on the air surface using pendant drop method on a KSV Cam 200 optical contact angle analyser at room temperature (KSV instruments Ltd., Finland). About 200 μL of de-ionised water was dropped onto the surface of each sample by means of a gas tight micro-syringe followed by capturing the images using the Windows based KSV-Cam software in order to monitor the contact angle.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was used in the characterisation studies to confirm the structural element of the individual polymers and their laccase assisted graft composites. Fig. 1 depicts the FT-IR spectra of P(3HB), BC, P(3HB)-g-BC and P(3HB)-g-BC*. For pure P(3HB) (Fig. 1), the peak at 3358 cm^{-1} refers to hydroxyl end groups while, peaks at 2926 cm^{-1}, and 2867 cm^{-1} are due to the stretching vibration of aliphatic C—H bond (Mansur, Sadahira, Souza, & Mansur, 2008). The high intensity of the 3358 cm^{-1} band in the graft composite showed an increase of hydrogen-bonding interactions between P(3HB) and BC at that distinct band region. This also implied the terminal hydroxyl groups of P(3HB) were consumed in reaction with hydroxyl groups of BC through laccase reaction. A sharp band observed at 1721 cm^{-1} is assigned to C=O stretching vibration. In the case of pure BC, a band at 2867 cm^{-1} is attributed to the aliphatic C—H stretching vibrational mode of BC. A sharp and steep band observed at 1056 cm^{-1} region is due to the stretching of the C—O—C linkage of cellulose molecules. Previously, FTIR spectroscopy has been used to prove the existence of hydrogen bonding interactions in the different composites based on cellulose/PHBV blends at different compositions (Hameed, Guo, Tay, & Kazarian, 2011).

3.2. Scanning electron microscopy (SEM)

Fig. 2 presents the morphological characteristics of the individual P(3HB), BC and grafted P(3HB)-g-BC and P(3HB)-g-BC* composites. SEM analysis revealed the clear nano-fibrils of BC as can be observed on the surface topographic scan in Fig. 2 (part B) while, the SEM micrograph of P(3HB)-g-BC shows that the P(3HB) completely filled the pores between the individual BC nano-fibrils and the vacant spaces between the BC sheets (Fig. 2 (part C). The SEM

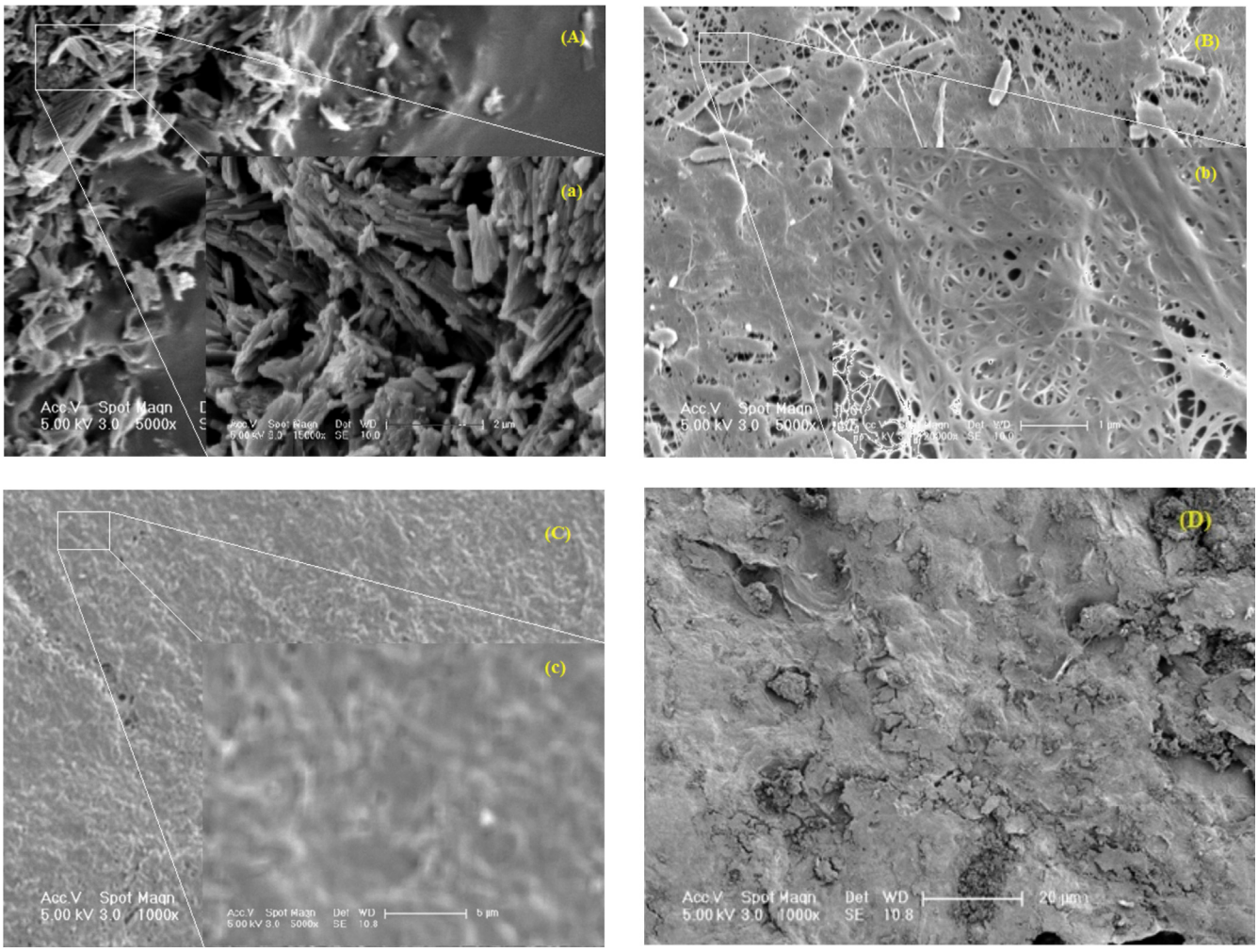

Fig. 2. Scanning electron microscopy of pure P(3HB) (A) and its magnified image (a), BC (B) and its magnified image (b), P(3HB)-g-BC (prepared using laccase) (C) and its magnified image (c) and P(3HB)-g-BC* (prepared in the absence of laccase) (D).

micrograph for P(3HB)-g-BC also provided evidence of the strong interaction between the BC nano-fibrils and the P(3HB) matrix, as shown by the good dispersion of P(3HB), without noticeable aggregates. The SEM photomicrograph of P(3HB)-g-BC* (composite prepared in the absence of laccase using buffer alone) in Fig. 2 (part D) shows that the P(3HB) in this composite tended to agglomerate into bundles and was unevenly distributed in the matrix. In an earlier study, Luo et al. (2009) have also observed a similar kind of rougher surface topography during the synthesis of composite films of P(3HB-co-3HHX) and P(3HB). Graft copolymerisation modified the surface morphology; physical, chemical, and microstructural characteristics of grafted materials, which in turn affects their bio-compatibility and biodegradability and this change in surface morphology supported the successful occurrence of graft polymerisation (Yu, Qin, Wang, & Zhou, 2012).

3.3. Differential scanning calorimetry (DSC)

The results obtained after DSC analysis are summarised in Table 1. In the DSC first heating scan, all of the tested samples reveal a glass transition temperature T_g from 9 °C to 30 °C and endothermal melting peak T_m of 174–198 °C, which is comparable with the T_m range of different thermoplastic PHAs polymers which are usually stored at room temperature (Dai, Lambert, Yuan, & Keller, 2008). The results showed a change in the T_g and T_m of the P(3HB)-g-BC composite as compare to that of the individual counterparts. In comparison to the pure P(3HB), the addition of cellulosic contents in the P(3HB)-g-BC, the crystallisation temperature (T_c) gradually reduces from 135 °C to 88 °C for the P(3HB)-g-BC (Table 1). Herein, the parameter T_c is used to measure the overall rate of crystallisation, and the smaller the T_c the greater the rate of crystallisation is. In an earlier study, Yu et al. (2012) has been reported that that the nucleation capability and crystallisation rate of neat PHBV can be greatly enhanced by achieving homogeneous incorporation of cellulosic based nanocrystals in the polymeric matrix.

3.4. Dynamic mechanical analyser (DMA)

The stress–strain behaviour in uniaxial tension of the individual polymers and grafted composites was recorded to measure the mechanical properties and the associated data along with the standard deviations are shown in Table 2. In the beginning at low strains the stress–strain behaviour of the BC and P(3HB)-g-BC was linear elastic. In comparison to the initial linearity behaviour a parabola like lap in the curve was recorded at a stress in the region of 70–105 MPa followed by a linear and fairly strongly strain-hardening region. Based on the data reported in literature this strain-hardening region is termed as plastic region (Henriksson, Berglund, Isaksson, Lindström, & Nishino, 2008). A simultaneous improvement was recorded in the mechanical properties of the laccase assisted graft composite prepared from P(3HB) in addition with BC as compare to its individual counterpart of P(3HB)

Table 1
DSC-thermal properties of the individual polymers *i.e.*, P(3HB) and BC, and their grafted composites *i.e.*, P(3HB)-g-BC and P(3HB)-g-BC*.

Sample ID	T_g (°C)	T_{onset} (°C)	T_m (°C)	T_{offset} (°C)	T_c (°C)	ΔH_m (J/g)
P(3HB)	9 ± 0.05	152 ± 0.41	174 ± 0.45	181 ± 0.58	135 ± 0.18	64 ± 0.02
BC	22 ± 0.20	172 ± 0.33	198 ± 0.38	207 ± 0.45	43 ± 0.05	92 ± 0.03
P(3HB)-g-BC	30 ± 0.09	164 ± 0.42	190 ± 0.29	202 ± 0.34	88 ± 0.05	73 ± 0.04
P(3HB)-g-BC*	23 ± 0.12	155 ± 0.39	177 ± 0.85	188 ± 0.42	118 ± 0.95	98 ± 0.26

Where, P(3HB) poly(3-hydroxybutyrate); BC, bacterial cellulose; P(3HB)-g-BC, poly(3-hydroxybutyrate) grafted bacterial cellulose (prepared using laccase); and P(3HB)-g-BC*, poly(3-hydroxybutyrate) grafted bacterial cellulose (prepared in the absence of laccase).

Table 2
DMA-mechanical properties of the individual polymers *i.e.*, P(3HB) and BC, and their grafted composites *i.e.*, P(3HB)-g-BC and P(3HB)-g-BC* measured from stress-strain curve.

Sample ID	Tensile strength (MPa)	Young's modulus (GPa)	Elongation at break (%)
P(3HB)	ND[a]	ND[a]	ND[a]
BC	139 ± 5.55	3.50 ± 0.39	9.22 ± 0.99
P(3HB)-g-BC	64.5 ± 2.33	0.98 ± 2.53	15.7 ± 2.65
P(3HB)-g-BC*	42.3 ± 1.31	2.41 ± 1.39	7.65 ± 0.33

Where, P(3HB), poly(3-hydroxybutyrate); BC, bacterial cellulose; P(3HB)-g-BC, poly(3-hydroxybutyrate) grafted bacterial cellulose (prepared using laccase); and P(3HB)-g-BC*, poly(3-hydroxybutyrate) grafted bacterial cellulose (prepared in the absence of laccase).

[a] Not detected.

because inherently high strength of BC allowed the mechanical properties of P(3HB)-g-BC composite to improve. In comparison to the pure BC a slight decrease in the Young's modulus of P(3HB)-g-BC was observed which is because of the incorporation of low strength P(3HB). On the other hand mechanical properties of the graft composite prepared from P(3HB) in addition with BC showed a commendable increase in the tensile strength (64.5 MPa), elongations at break (15.7%) and Young's modulus (0.98 GPa) as compare to the P(3HB) alone which was too brittle to measure any of the above said characteristics. Similar kind of behaviour was also observed for the tensile strength of the individual polymers and grafted composites. Unlike native BC which is flexible, P(3HB) is crystalline and brittle in its nature, therefore, has much lower mechanical values. Characteristic XRD diffractogram (Fig. 3A–D) shows that the P(3HB) used in this study lack the amorphous regions and flexibility found in BC. In the present study, as concluded from the T_g and XRD measurements, the interactions between the P(3HB) and BC, if any, alter the thermal and physical properties of the P(3HB) and BC, respectively. Floros et al. (2012) obtained similar results where there was an increase in the Young's modulus of the P(3HB-co-4HB)/NC nanocomposites with the increase in concentration of NC.

3.5. X-ray diffraction (XRD)

Characteristic XRD diffractogram of the pure P(3HB), BC, P(3HB)-g-BC and P(3HB)-g-BC* composite was given in Fig. 3. The changes in the crystallinity of the pure P(3HB) caused by incorporating the BC were investigated by X-ray diffraction. For the BC, a main scattering intensity peak can be identified at 2θ value of 21.2° as shown in Fig. 3, which is assigned to the reflexion planes of cellulose I (Tokoh,

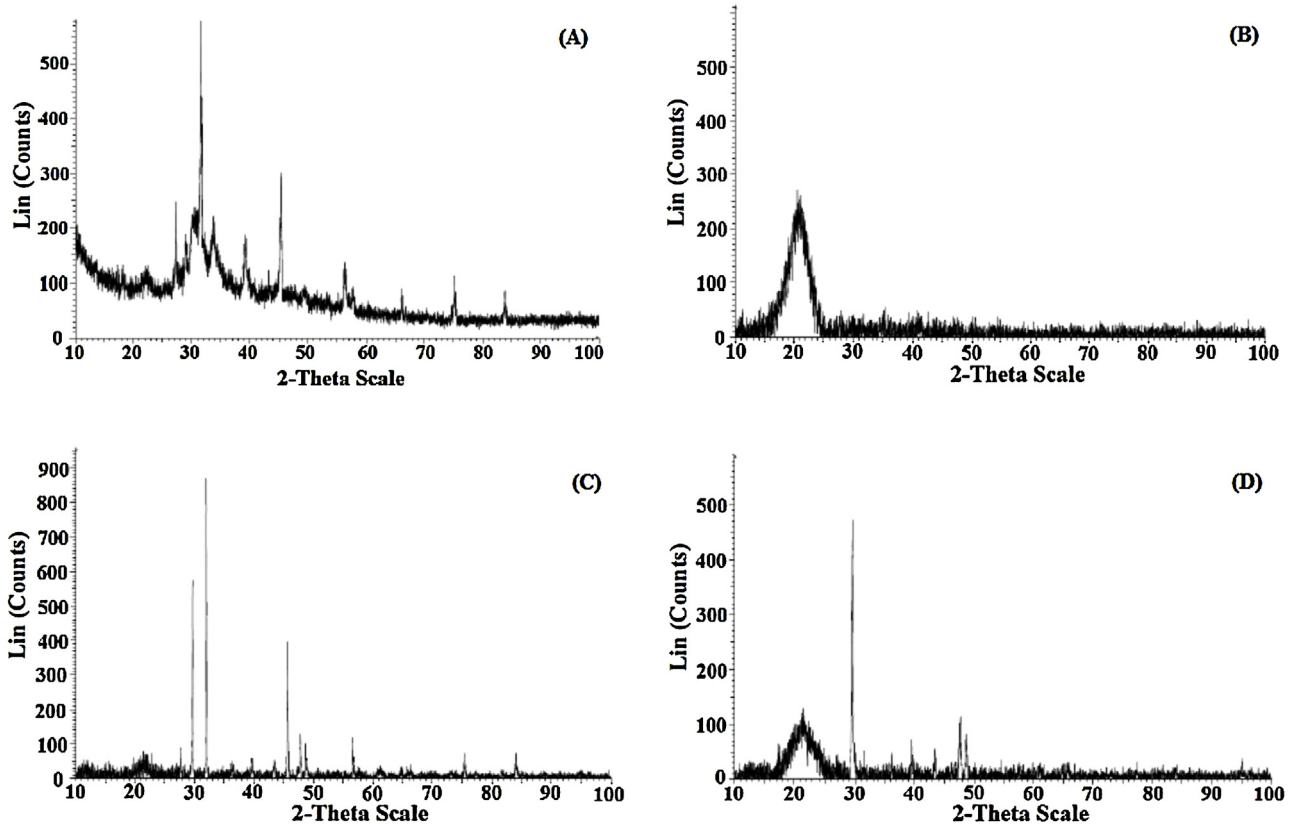

Fig. 3. Typical diffractogram of the individual polymers and their graft composites taken from XRD; (A) P(3HB); (B) BC; (C) P(3HB)-g-BC and (D) P(3HB)-g-BC*.

Table 3
Proposed applications of bacterial cellulose based "green" graft composites.

Materials	New/improved functionalities	Potential applications	Reference
P(3HB) and BC	Thermo-plasticity, physical and mechanical strength, biodegradability	Bio-plastics	Lao et al. (2007)
P(3HB) and BC	Biocompatibility, thermo-plasticity, physical and mechanical strength	Tissue engineering	Bettinger (2011)
P(3HB) and BC	Biocompatibility, biodegradability, non-toxicity	Medical/drug delivery	Alves and Mano (2008)
P(3HB) and BC	Biocompatibility	Biomarkers/biosensors	Tastan, Önder, and Kok, (2011)

Takabe, Fujita, & Saiki, 1998). In the X-ray diffraction pattern of the P(3HB)-g-BC composite (Fig. 3), a change in the characteristic peaks of both P(3HB) and BC was observed, which means the reduction in the crystalline and amorphous nature of the P(3HB) and BC, respectively. The reduction in the crystallinity is mainly because of the new cross-linking of P(3HB) within the BC backbone that changes the morphology and destroys the crystallites. Compared with pure the P(3HB), P(3HB)-g-BC has some changes at the 2θ values and the peaks of the P(3HB) in the composite [P(3HB)-g-BC] were sharper and clearer than those in the pure P(3HB). The results obtained through XRD analysis evidently support that the degree of crystallisation behaviour of the P(3HB) can be greatly reduced by achieving homogeneous incorporation of amorphous cellulose into the polymeric matrix. Furthermore, there might be an interaction between the enzymatically treated cellulose and P(3HB), the motion of the P(3HB) side chains is restricted and subsequently causes the reduction of crystallinity in P(3HB)-g-BC.

3.6. Water contact angle (WCA)

Fig. 4 illustrates the contact angles and surface tension characteristics of the individual polymers [(P(3HB) and BC)] and their resulting graft composites [P(3HB)-g-BC and P(3HB)-g-BC*], respectively determined by deionised water *via* the static contact angle measurement. It was found that P(3HB) has larger water contact angle *i.e.*, 68° whereas comparative to the P(3HB), BC has lower contact angle value *i.e.*, 15°, which means BC has very high hydrophilicity. For P(3HB)-g-BC with more hydroxyl groups of side chains exhibited more hydrophilic features than its individual P(3HB) counterpart which was hydrophobic in nature. It has been reported in literature that the contact angle of polymer surface can be decreased by introducing hydrophilic groups, such as hydroxyl, carbonyl, and carboxyl group. On the other hand, the surface tension properties of the pure P(3HB) was about 20.5 mN/m which increased up to 59.2 mN/m with the reinforcement of hydrophilic BC into the P(3HB) matrix in case of P(3HB)-g-BC grafted composite. Wettability evaluated through the measurement of the contact angle of a liquid on a surface is a sensitive way to measure the hydrophilic/hydrophobic features of a material (Pertile, Andrade, Alves, & Gama, 2010). It can be expected that the P(3HB)-g-BC composite with high hydrophilicity is more suitable for cell adhesion and proliferation than pure P(3HB). Similar results were observed by Basnett et al. (2012) where there was an increase in the hydrophilicity of the P(3HO)/BC (acetylated bacterial cellulose) blend films compared to the neat films due to the incorporation of hydrophilic acetylated bacterial cellulose.

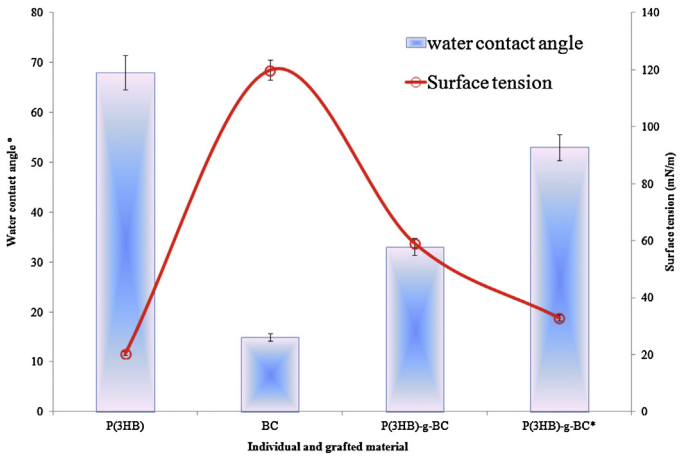

Fig. 4. Static water contact angle and surface tension measurements of the individual polymers *i.e.*, P(3HB) and BC, and their grafted composites *i.e.*, P(3HB)-g-BC and P(3HB)-g-BC*.

4. Conclusions

In conclusion, the results obtained in the present study demonstrates that there were good interactions between P(3HB) and BC. The newly synthesised composites are expected to find potential applications in various industrial and bio-medical or pharmaceutical sectors, where bio-compatible, bio-degradable, and environmentally friendly polymer composites with improved properties are in demand. Potential applications, on the basis of new or improved functionalities of the grafted polymers, are summarised in Table 3. The present research is a first step in the biopolymers modification by using the technique based on enzymatic grafting. To date, there is no report in literature explaining the laccase assisted grafting of P(3HB) and BC. The synthesis of laccase-assisted green composites opens the doors to effective polymer reinforcement without the environmental and economic restraints of petroleum-based alternatives. In conclusion, the emerging laccase bio-grafting will be one of the most important methods in the foreseeable future of biotechnological techniques in this area.

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