Surface modification of natural fibres using bacteria: Depositing bacterial cellulose onto natural fibres to create hierarchical fibre reinforced nanocomposites

Marion Pommet[1a], Julasak Juntaro[1a], Jerry Y.Y. Heng[1b], Athanasios Mantalaris[1c], Adam F. Lee[2], Karen Wilson[2], Gerhard Kalinka[3], Milo Shaffer[4] and Alexander Bismarck[1a*]

[1] Department of Chemical Engineering,

[1a] Polymer & Composite Engineering (PaCE) Group

[1b] Surfaces and Particle Engineering Laboratory

[1c] Biological Systems Engineering Laboratory

Imperial College London, South Kensington Campus, London SW7 2AZ, UK

[2] Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK

[3] Federal Institute for Materials Research and Testing (BAM), Division V.6, Unter den Eichen 87, 12205 Berlin, Germany

[2] Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

[*] Corresponding author: a.bismarck@imperial.ac.uk

Abstract

Triggered biodegradable composites made entirely from renewable resources are urgently sought after to improve materials recyclability or be able to divert materials from waste streams. Many bio-based polymers and natural fibres usually display poor interfacial adhesion when combined in a composite material. Here we propose a way to modify the surfaces of natural fibres by utilising bacteria (*Acetobacter xylinum*) to deposit nanosized bacterial cellulose around natural fibres which enhances their adhesion to renewable polymers. This paper describes the process of modifying natural fibres with bacterial cellulose through their use as substrates for bacteria during fermentation. The modified fibres were characterised by scanning electron microscopy, single fibre tensile test, X-ray photoelectron spectroscopy and inverse gas chromatography in order to determine their surface and mechanical properties. The practical adhesion between the modified fibres and the renewable polymers cellulose acetate butyrate and poly (L-lactic acid) was quantified using the single fibre pullout test.

Keywords: Bacterial cellulose; natural fibre; surface modification; interfacial shear strength, hierarchical nanocomposite

Introduction

A composite is a structural product made of two or more distinct materials whose engineering performance is by far exceeding those of any individual component. Composites made with synthetic fillers such as glass or carbon fibres are nowadays extensively used for many applications, for instance in sport, transport, automotive and aerospace. Their success is due to their specific mechanical properties, based on a strong interaction between the different components and their durability. However, it is consequently usually impossible (or at least very difficult) to separate the different components again[1-3] and, therefore, to recycle the composites, which generates end-of-life disposal problems.

Landfill, through which 98% of composite waste is disposed off (2003 figure)[4], will become prohibitively costly through the new European waste legislation in most European Union (EU) member states[5]. The EU end-of-life vehicles directive, applying to all passenger cars and light commercial motor vehicles, will allow only an incineration quota of 5% for disused cars by 2015[6]. Another EU legislation, the Waste Electrical and Electronic Equipment (WEEE) Directive[7], also affects composite and polymer manufacturers by forcing them to provide for recycling of their products. As a result of these new legislations, both manufacturers and end-users will need to move away from traditional materials and will require new strategies for environmentally and economically viable materials. Truly green biodegradable composites made entirely from renewable agricultural resources could offer a unique alternative to address these issues for materials used in low load bearing applications[8].

A broad range of renewable or partially renewable polymers, such as cellulose acetate butyrate (CAB), polylactic acid (PLA), or Dupont's Sorona®, is now commercially available[9]. Alternative fillers such as natural fibres have already been explored for certain applications[10]. Advantages of natural fibres are their low cost, low density, abundance, renewability and (potentially their) biodegradability. They also display high specific stiffness and strength as well as acoustic and thermal insulation properties due to their hollow and cellular nature. Their drawbacks arise mainly due to their inconsistency in their dimensions and mechanical properties, their water sensitivity and their low compatibility with many hydrophobic polymeric matrices[11]. Bad or no adhesion at the interface (Fig. 1) between the two components will lead to a composite with poor mechanical properties since the stress transfer to the reinforcing phase through the matrix phase will not be effective. To improve the interaction between natural fibres and the matrix, it is necessary to modify the natural fibres or the bio-based polymers in order to compatibilise them, which is required for the design of truly green composites that can compete with conventional composite materials, such as glass

fibre reinforced polypropylene. Chemical modifications such as silanisation of natural fibres[12 - 17] or anhydride grafting of bio-based polymers[18] have been studied and found to lead to increased composite properties. However, these modifications affect the *green image* of the final composites.

Recent studies pointed out that nano-scale cellulose is an interesting green reinforcing agent for the design of nanocomposites[19 - 24]. Cellulose microfibrils can be extracted from wood or many other plant based materials but pulping and bleaching processes are not environmentally friendly[25]. Cellulose whiskers can also be extracted from tunicate, a sea animal[26]. Bacterial or microbial cellulose is produced by certain bacteria belonging to the genera *Acetobacter*, *Agrobacterium*, *Alcaligenes*, *Pseudomonas*, *Rhizobium* or *Sarcina*, the most efficient producer of bacterial cellulose being *Acetobacter xylinum*[25]. *Acetobacter xylinum*, an obligate aerobe, produces extracellular cellulose microfibrils to provide a firm matrix that floats and, therefore, allows the embedded bacteria to stay in close contact with the atmosphere. The produced cellulose pellicles play a great role in promoting colonisation of the cells on the substrate and provide protection against competitors. Cellulose pellicles were also observed to protect *Acetobacter xylinum* cells from UV light[27].

Recently, Guhados *et al.*[28] have measured the elastic modulus of single bacterial cellulose fibril using atomic force microscopy to be 78 GPa, which is much higher than those of natural fibres (generally less than 30 GPa)[29] and is in the same order as that of glass fibres (70 GPa)[30]. This makes bacterial cellulose a very promising green nano-reinforcement. Moreover, the very good mechanical properties obtained for some cellulose-reinforced renewable nanocomposites[23] prompt us to assume that the interfacial adhesion between bacterial cellulose and renewable polymers should be good.

Inspired by nature, creating very complex hierarchical structures by assembly of molecules of different sizes where high mechanical resistance is needed, such as in plant cell walls, animal shells and bones, we propose an alternative way of modifying natural fibre surface. A hierarchical structure was produced by cultivating cellulose-producing bacteria in presence of natural fibres, which resulted in significant coverage of the fibre surfaces by bacterial cellulose. This green modification is aimed at improving the interfacial adhesion to bio-based polymers and might lead to truly green fibre reinforced hierarchical nano-composites with enhanced properties and much better durability.

Materials and Methods

Materials

Loose hemp fibres and mats were kindly supplied by Hemcore Ltd. (Hertfordshire, UK) and loose sisal fibres and mats by Wigglesworth & Co. Ltd. (London, UK). The cellulose producing bacteria strain *Acetobacter xylinum* BPR2001 (ATCC no. 700178) was purchased from LGC Promochem (Middlesex, UK). It was selected because of its high cellulose production capability under agitated conditions[31]. Cellulose acetate butyrate (CAB-500-5 with 51% butyryl content, 4% acetyl content and 1% hydroxyl content, M_w = 57000 g/mol, 1.14-1.28 g/cm^3) was supplied by Eastman Chemical Co. (Kingsport, Tennessee, USA). All other chemicals used were purchased from Sigma-Aldrich Company Ltd. (Dorset, UK).

Fibre modification in small scale static cultures

Loose sisal or hemp fibres (0.5 g, 10 cm long) or fibre mats (4 × 4 cm) were put in 250 ml Erlenmeyer flasks containing 90 ml of culture medium which composed of 50 g/L fructose, 5 g/L yeast extract, 5 g/L peptone, 2.7 g/L Na_2HPO_4, and 1.15 g/L citric acid. The medium was formulated after H&S medium[32], but with higher content of sugar and with fructose in place of glucose. This formulation was found to promote the production of bacterial cellulose with stable pH. After autoclaving at 121°C for 20 min, the flasks were inoculated with 10 ml of a 3 day old broth of a previous culture of *Acetobacter xylinum* BPR2001. The fermentation was conducted under agitated conditions on a shaking plate (150 rpm) in an environmental chamber at 30°C for one week.

Fibre modification in an agitated 5 L fermentor

Natural fibres were also modified in a 5 L bioreactor (BioFlo II, New Brunswick Scientific, Hertfordshire, UK) with air supply and pH regulation to pH = 5. The agitation was provided by two turbines. A round stainless steel cassette was designed and incorporated around the impeller shaft in order to store plant fibres or fibre mats inside the fermentor during the fermentation (Fig. 2). The cassette was not fixed to the impeller shaft so that it could rotate independently of the agitation speed, with two stoppers preventing it from moving along the shaft. Alternative to the use of the cassette, loose fibres (50 g, 1 cm long) were directly added to in the culture medium. The fermentor was autoclaved with 3.5 L of medium (same composition as above) with the filled cassette or loose fibres dispersed in the medium. The temperature of the fermentor was regulated to be 30°C and the rotation speed

set to 750 rpm. The fermentor was inoculated with 500 ml of a 3-day old broth of a previous culture of *Acetobacter xylinum* BPR2001.

Extraction of the modified natural fibres

After the fermentation, the modified natural fibres were purified in 0.1 M NaOH at 80°C for 20 min in order to remove all microorganisms, medium components and soluble polysaccharides[31]. After filtration, they were then thoroughly washed in distilled water until neutral pH. The weight change of the fibres before and after any modification (step) was determined. In order to improve the accuracy of the measurement fibre sample size of about 4 g was used for the procedure.

Morphology of the modified fibre surface

Scanning Electron Microscopy (SEM) was used to study the surface morphology of the fibres. SEM was carried out using a LEO 1525, operating at 7 kV. Samples were fixed to aluminium stubs with carbon tape, then vacuum-dried and sputter coated with approximately 10 nm of gold particle for 2 min at 20 mA (Emitech Ltd K550, Ashford, UK).

Mechanical properties of the modified fibres

Single fibre tensile tests were performed on fibres following ASTM 3379-75. The specimens were conditioned at 20°C and 50% relative humidity for one week before testing. The tensile tests were conducted using an Instron universal material testing machine (Instron 5584, Instron Ltd, UK), using a gauge length of 20 mm and a tensile speed of 1 mm/min. At least ten fibres per sample were tested.

Surface properties of the modified fibres: Surface energy of natural fibres

The surface energy of a material can be described by the sum of a dispersive component (γ_s^d), accounting for the capacity of the surface to exchange London or dispersive interactions, and a specific component referring to all other possibilities of interactions (induction, dipole and hydrogen bond). The dispersive component is called the non-specific component of the surface energy, since London interactions always intervene irrespective of the partners brought into contact. The specific component cannot be simply measured. Among the specific interactions, acid-base interactions seem to play a key role in the interfacial interactions between the components of a composite[33-35].

To measure the surface energy, fibre samples were packed into pre-silanated glass columns (4 mm ID) with silanated glass wool end frits. Samples were pretreated at 30°C, 0% relative humidity for 2 h to remove any residual moisture. A series of alkane vapours (decane, nonane, octane and heptane) was selected and used as probes for the dispersive surface free energy of the fibres. Methane was used as a non-interacting probe to determine the dead-time of the column. The injected probes were at infinite dilution (4%) for a peak maximum analysis and chromatograms were obtained with an SMS-iGC 2000 (Surface Measurements Systems Ltd., London, UK). The dispersive component of the surface energy was calculated according to the method proposed by Schultz *et al.*[36] using the SMS-iGC analysis software (version 1.2, Surface Measurements Systems Ltd., London, UK). The acid-base numbers were obtained from polar probes (acetone, ethanol, acetonitrile and ethyl acetate) based on the Gutmann analysis[37].

Surface properties of the modified fibres: Surface composition of natural fibres

X-ray photoelectron spectroscopy (XPS) was performed using an Axis HSi spectrometer, equipped with Mg K_α anode and charge neutraliser. Survey spectra and high resolution scans were acquired with pass energies of 160 and 20 eV respectively. All spectra were energy referenced to the valence band and C 1s CH_x environment at 285 eV. Surface compositions were determined by quantification of high resolution regions using appropriate elemental sensitivity factors for O 1s (0.736), N 1s (0.505) and C 1s (0.318) regions.

Interaction of the modified fibres with renewable polymers: Interfacial shear strength (IFSS)

Single fibre pull-out tests were performed in order to determine the apparent interfacial shear strength (τ_{IFSS}), as measure of the practical adhesion between the (bacterial cellulose modified) natural fibres and PLLA and CAB. A single fibre was partially embedded to a pre-determined length between 50-150 μm in a PLLA or CAB melt droplet using a home made apparatus[38]. Polymer powder was placed on an aluminium sample carrier, heated to and held well above the melting temperature while the fibre was penetrated into the melt. Afterwards the sample was cooled to room temperature using an air stream. The single fibre was then fixed to a screw platform using super glue. The screw platform is attached to a piezo-motor fixed on a high stiff frame in order to avoid energy storage in the free fibre length between the matrix surface and the clamping device. The fibre was loaded at a speed of 0.2 μm/s, while the force was recorded throughout the experiment using a computer[39]. τ_{IFSS}

was calculated from the maximum pull-out force F_{max} required to trigger the debonding of the embedded natural fibre from the matrix using the following equation:[40]

$$\tau_{IFSS} = \frac{F_{max}}{P_f L} \quad (1)$$

where P_f is the perimeter of the fibre and L is the embedded fibre length. L was determined from the force-displacement curve, i.e. it is the displacement when the force dropped to zero. The fibre perimeter P_f was directly determined from the imprint of the fibre on the matrix droplet post pullout using SEM. Maximal loads were then plotted as a function of embedded area and the slope of the graphs was taken as τ_{IFSS}. A minimum of 6 measurements were performed per sample type to determine τ_{IFSS}.

Results and Discussion

Fibre modification in small scale cultures

We first developed a method of attaching bacterial cellulose to natural fibre surfaces at a small scale. The objective was to cultivate cellulose producing bacteria on plant fibres used as growing support. Bacterial cellulose being an extracellular product of the bacteria would grow on the fibre surface and, provided that suitable interactions occurred between the two, bacterial cellulose would attach to the natural fibre surfaces. Consequently the natural fibre surfaces would be modified at a nanometre scale.

The bacteria strain *Acetobacter xylinum* BPR2001 was found to grow preferably on the natural fibre surface rather than freely in the medium. The natural fibres provide ideal substrates for the bacteria because of their hydrophilic and rough surface. The fermentation process in presence of natural fibres therefore led to the formation of bacterial cellulose-based pellicles preferentially around the plant fibres (Fig. 3). After the NaOH extraction, a white cellulose layer could still be seen around the modified natural fibres, which pointed the strong interaction between bacterial cellulose and the fibre surface; this could be because of the high self-affinity of cellulosic materials[27]. The large number of hydroxyl groups at the surfaces of the substrate and of the bacterial cellulose will help promoting hydrogen bonding between them. It is also possible that the bacterial cellulose fibril could root through the porous natural fibre.

In order to determine how much bacterial cellulose was deposited around the natural fibres the weight before and after each modification step was recorded (Table 1). The weight of the fibres was measured after each individual treatment step, i.e. after the fibres were autoclaved in the medium without fermentation, autoclaved in the medium without

fermentation followed by NaOH extraction (blank control), autoclaved in the medium with fermentation, and autoclaved in medium with fermentation and NaOH extraction. Both hemp and sisal lose weight (7% and 4%, respectively) during the sterilisation heat treatment at 121ºC for 20 min in the autoclave. Water soluble components are leached out form the fibres during this process. The consecutive extraction in NaOH at 80ºC caused a total weight loss of approximately 10% to the fibres (blank control). This is because NaOH extraction results in the removal of non-cellulose compounds from the fibres[11]. On the other hand, after bacterial fermentation in presence of the fibres, the fibres gain weight mainly because of the bacteria adhering to the fibres and deposition of cellulose and polysaccharides. After the NaOH extraction the modified fibres still gained between 5 - 6 % weight as compared to the blank control. This weight gain is due to the attachment of bacterial cellulose to the modified fibres.

SEM micrographs of the surface of hemp fibres before (Fig. 4a) and after (Fig. 4b) the modification with bacterial cellulose clearly show the presence of bacterial cellulose all around the fibre surface. Bacterial cellulose nanofilaments of 50 to 100 nm in diameter completely covered the hemp fibre surface. The cellulose fibrils were randomly oriented around the natural fibres.

The same bacterial cellulose fermentation was performed in presence of sisal fibres. The surface of sisal was originally quite smooth (Fig. 5a). In this case, however, the bacterial cellulose pellicle appeared to be much less attached to the sisal fibres, which left parts of the fibres covered only by little bacterial cellulose nanofibrils (Fig. 5b). In order to improve the compatibility of the bacteria and the produced bacterial cellulose to the natural fibre substrate, waxes and other organic compounds that formed a protecting hydrophobic layer around sisal were removed using Soxhlet extraction of the fibres in acetone for 1 h, which led to fibres that would be much more readily wetted by water[41, 42]. The fermentation process was then conducted using these pre-treated sisal fibres and resulted in full coverage of the acetone-treated sisal fibre surface with bacterial cellulose (Fig. 5c), similar to hemp (Fig. 4b).

It can be assumed that this method of natural fibre surface modification can successfully be applied to any natural fibre, provided that its surface is hydrophilic enough between enable interaction to the cellulose producing bacteria, the produced cellulose and the natural fibres.

Large scale fibre modification in an agitated 5 L fermentor

Having succeeded in the small scale surface modification of natural fibres, the method was scaled up to work in a 5 L fermentor. The modification was first performed on natural

fibre mats that were inserted into the cassette specifically designed (Fig. 2). The bacteria were found to mainly grow around the cassette (Fig. 6a) but much less inside (Fig. 6b). This was possibly due to the fact that the conditions inside the cassette might be too anaerobic despite the air flow provided just underneath.

The fermentation was then conducted using loose fibres freely suspended in the medium. The fibres were cut to a length of 1 cm so that they did not entangle around the turbines. However, even under these conditions the loose fibres tended to agglomerate, resulting in the growth of bacterial cellulose pellicles on the fibre surface and around the agglomerates (Fig. 6c). The modified fibres could not be isolated easily after the fermentation process. However, even the fibres inside the agglomerates were partially covered by bacterial cellulose (Fig. 7), which was not observed in small scale shaken flask cultures (Fig. 5c).

Mechanical properties of the modified fibres

In order to determine the impact of our fibre surface modification procedure on the mechanical properties of the modified fibres, single fibre tensile tests were performed. The mechanical fibre properties were determined after each individual treatment step in the same way as for the weight gain measurements. In the case of sisal fibres, no significant difference in mechanical properties can be observed (Table 1). Neither the extended exposure to the culture medium, nor the NaOH extraction, nor the procedure of attaching bacterial cellulose to the sisal surfaces affects the tensile properties of the fibres. This result is encouraging for the production of composite materials, since the reinforcing potential of the fibres will remain intact. However, in the case of hemp fibres, a significant decrease in the Young's modulus and, to a lower extent, in the tensile strength of the fibres was observed after the exposure to the culture medium without bacteria. The fermentation process further affected the mechanical properties of the hemp fibres. It could be seen by the naked eye that the processed technical hemp fibre bundles split into finer sub-fibres, i.e. finer technical or elementary fibres significantly affecting the mechanical properties of the fibres. This phenomenon was due to the fact that the structure of technical bast (hemp) fibre bundles is less cohesive than that of leaf (sisal) fibres.

Surface properties of the modified fibres

Surface modification of natural hemp and sisal fibres was first investigated by XPS. Table 2 shows the surface composition of fibres before and after the fermentation. Hemp and sisal fibres are mainly composed of cellulose, hemicellulose, lignin and pectin[29]. Pure

cellulose fibres would be expected to have an O/C ratio around 0.8. Our observed ratios of 0.1 and 0.3 for untreated hemp and sisal respectively, were thus consistent with them either being coated with hydrocarbon-rich waxy coatings or containing a large fraction of lignin at the surface. However, please note that pre-acetone extraction of sisal fibres did not affect their surface composition. Following the surface modification using bacteria the O/C ratio rose significantly for both hemp and sisal fibres, consistent with the formation of oxygen rich cellulose-like deposits on the surface.

More detailed analysis of the surface composition of treated fibres is shown in the high resolution C 1s XP spectra shown in Fig. 8. It can be seen that after the bacterial surface modification of both fibres the number of C-OR functionalities increases significantly. These spectra can be interpreted from a consideration of the basic building blocks of polymeric molecules like cellulose and lignin shown in Scheme 1. A number of C chemical environments would be expected in cellulose, including C-C-OH, C-OH, C-O and O-C-O. In contrast the lignin building blocks contains aromatic groups and is expected to be much less polar, with mainly CH_x and C-OR groups present in either a 3:1 or 3:2 ratio depending on the precise composition of the lignin shown in Scheme 1. Table 3 gives the integrated areas from the fitted spectra in Fig. 8. Natural hemp and sisal both had high CH_x contents, with CH_x:CO_x ratios of 4:1 and 3:2 respectively, consistent with the presence of lignin at the fibre surface. Following the fermentation the C-O-C and O-C-O content of both materials increased significantly at the expense of CH_x, suggesting the successful deposition of cellulose-like material on the surface of both hemp and sisal fibres, and attenuation of the underlying lignin-like coating.

The dispersive part of the fibre surface energy, γ_s^d, was presented in Table 4, as well as acid (K_A) and base (K_B) numbers, describing respectively the electron acceptor or donor capacities of the surface. γ_s^d obtained for natural fibres are similar to values found in the literature[42~~41~~,43,44]. The high value obtained for bacterial cellulose can be related to its high degree of crystallinity compared to plant derived cellulose. Papirer *et al.*[45] have shown that the surface energy of cellulose is a function of the degree of crystallinity by studying various cellulose samples differing in their crystallinity. Bacterial cellulose was found to have a high K_B, displaying high electron donor ability. These differences should allow us to verify the presence of bacterial cellulose at the surface of the modified fibres, as well as provide some estimates of the covering density of the modified fibres natural fibres by deposited bacterial cellulose.

The bacterial cellulose modification led to a small increase of γ_s^d in sisal fibre, in comparison to acetone-treated sisal (Table 4). However, no increase was detected in γ_s^d obtained for hemp fibres. The fact that the surface energy of hemp fibres remained unchanged after attaching a high surface energy component to them could indicate either a low coverage of bacterial cellulose or the re-deposition of compounds extracted from the fibres following post-NaOH extraction onto the bacterial cellulose-modified fibre surface. To investigate this re-deposition, a pre-treatment of hemp with NaOH was carried out with the intention of removing such extractable compounds prior to the fermentation. This step also ensured that no unwanted deposit will form above the bacterial cellulose layer due to the post-NaOH extraction of the fibre following the fermentation. Indeed, the γ_s^d of the NaOH-pretreated, bacterial cellulose-modified sisal and hemp were found to be similar to that of pure bacterial cellulose, indicating a better attachment of bacterial cellulose to the pre-treated fibres.

K_B of both fibres were found to approach that of bacterial cellulose following the fermentation. However, after the NaOH-pre-treatment we did not observe the same trend for K_B. Further investigation is still required to better understand these variations in K_B.

Adhesion between the modified fibres and CAB and PLLA

The adhesion between the modified hemp and sisal fibres and the renewable matrices CAB and PLLA was quantified using the single fibre pull-out test. The apparent IFSS as measure of the practical adhesion was determined using Eq. 1. The IFSS of the bacterial cellulose modified fibres increased significantly as compared to the unmodified sisal (Table 5). After optimising the modification conditions for sisal it was impossible to determine the IFSS because the internal fibre structure (a composite itself) failed rather than the fibre matrix interface. SEM images taken of both the pulled-out fibre fragment and the cavity matrix (Fig. 9b) clearly show that the outer layer of the bacterial cellulose modified acetone washed sisal fibres remained adhered to the matrix, i.e. the fibre failed cohesively. In contrast, all the other fibres exhibited clean, smooth surfaces after pull-out (Fig. 9a), implying an adhesive failure at the fibre-matrix interface. Cohesive fibre failure occurs when the interfacial adhesion exceeds the adhesion between the subfibres which form the sisal fibre[18,19]. It should be noted that the improvement in the fibre/matrix interaction cannot be attributed to the acetone treatment; it results in a decrease of the IFSS (Table 5). In addition we also found an improved interaction between the modified fibres and a CAB matrix. The IFSS of both hemp and sisal to a CAB matrix also significantly improves after the bacterial cellulose modification (Table 5 and Fig.

10). In order to determine the average τ_{IFSS} between the modified fibres and polymer the maximum pull-out force was plotted as function of the embedded fibre area. Fig. 10 shows exemplarily the pull-out data for the hemp and sisal fibres from CAB. The gradient corresponds to the apparent interfacial shear strength. The steeper slope for the bacterial cellulose modified fibres indicates a stronger apparent adhesion. This stronger interface presumably arises from the increase in roughness associated with the presence of nanoscale cellulose on the surface and the entanglement between the bacterial cellulose fibrils and polymer molecules. Strong interactions are expected due to the potential for hydrogen-bonding between the hydroxyl groups present on the modified fibre surface and in CAB, and the carbonyl groups in PLLA. Fibre roughening, on the other hand, has been shown to improve adhesion in a wide range of fibre composite systems; of particular relevance is the attachment of carbon nanofibres onto conventional carbon[46,47] or silicon carbide[48] fibres. The improved adhesion will enhance the stress transfer efficiency between the two phases; in turn an improvement in composite performance was found[49~~48~~].

Conclusion

We describe a simple method to combine common natural fibres and nanosized bacterial cellulose. We propose a *green* way to modify natural fibres by attaching bacterial cellulose nanofibrils to the surfaces of natural fibres by using them as substrate during the fermentation process of bacterial cellulose. The persistence of the modification after NaOH extraction shows the strength by which bacterial cellulose is attached to the natural fibres. The adhesion between bacterial cellulose nanofibrils and natural fibres is possibly related to a high number of hydrogen bonds formed between the bacterial cellulose and the natural fibre. The adhesion between the deposited cellulose and natural fibres can be enhanced by pre-treating the fibres by a solvent extraction to remove the hydrophobic compounds from the fibre surface.

Simple weight gain measurements before and after the modification show that about 5 – 6% bacterial cellulose adheres to the fibres as a result of the bacterial modification procedure. SEM micrographs confirm the presence of attached bacterial cellulose on the surfaces of natural fibres. IGC confirms the presence of bacterial cellulose on fibres, which leads to an increase in the dispersive component of the surface energy γ_s^d of the natural fibres because of the attachment of the higher surface energy bacterial cellulose to the fibres. γ_s^d of pure bacterial cellulose is 61 mJ/m^2. However, an appropriate pre-treatment of the natural

fibres to be used as substrate during the bacterial cellulose fermentation process needs to be undertaken to avoid re-deposition of extractable compounds onto the surface. The mechanical properties of sisal fibres were not affected by the modification process, contrary to those of hemp fibres. The exposure of the hemp fibres to the fibre surface modification procedure causes a drastic loss of fibre strength as well as Young's modulus, which is due to a further separation of the technical fibres in to smaller fibres because of the non-cohesive structure of bast fibres.

The deliberate introduction of nanosized bacterial cellulose provides a new means to control the interaction between the modified fibres with a polymer matrix. The modified fibres were incorporated into PLLA and CAB, to obtain a new class of model hierarchical composite. The attaching approach results in a significantly increased interfacial adhesion to both polymers. The hierarchical structure obtained (with sisal fibres) will consequently lead to greatly improved mechanical performance of composites[49].

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Table 1: Weight changes and mechanical properties of natural and modified fibres.

Sample	Weight Change (%)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)
Natural sisal fibres	0	15.0 ± 1.2	342 ± 33	2.9 ± 0.1
Sisal fibres after sterilisation at 121ºC for 20 min in medium without bacteria	- 7.2	13.8 ± 1.8	352 ± 42	5.4 ± 1.0
Sisal fibres after sterilisation at 121ºC for 20 min in medium without bacteria and after NaOH extraction at 80ºC *(Blank control)*	**- 10.1**	12.2 ± 1.3	343 ± 21	4.8 ± 0.6
Sisal fibres after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	+ 2.0	12.5 ± 1.0	324 ± 33	4.5 ± 0.4
Sisal fibres after sterilisation at 121ºC for 20 min in medium, modification with bacterial cellulose and after NaOH extraction at 80ºC	**- 3.7**	12.0 ± 0.9	310 ± 32	4.1 ± 0.5
Natural hemp fibres	0	21.4 ± 2.0	286 ± 31	2.0 ± 0.2
Hemp fibres after sterilisation at 121ºC for 20 min in medium without bacteria	- 4.0	13.5 ± 2.7	263 ± 22	2.7 ± 0.2
Hemp fibres after sterilisation at 121ºC for 20 min in medium without bacteria and after NaOH extraction at 80ºC *(Blank control)*	**- 11.1**	15.1 ± 1.7	224 ± 39	2.5 ± 0.2
Hemp fibres after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	+ 2.0	8.8 ± 0.7	171 ± 11	2.9 ± 0.2
Hemp fibres after sterilisation at 121ºC for 20 min in medium, modification with bacterial cellulose and after NaOH extraction at 80ºC	**- 5.7**	8.0 ± 0.6	130 ± 12	2.3 ± 0.2

Table 2: Surface composition of hemp and sisal fibres after fermentation.

Sample	Surface composition / at.-%			
	O	**N**	**C**	**O/C**
Natural hemp fibre	10.8	0.4	88.8	0.1
Hemp fibres after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	22.5	1.0	76.5	0.3
Natural sisal fibre	24.6	1.2	74.2	0.3
Sisal fibre acetone extracted	24.3	0.9	74.8	0.3
Acetone treated sisal fibre after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	34.6	1.7	63.7	0.5

Table 3: C 1s component intensities of hemp and sisal fibres after bacterial cellulose modification.

Sample	Surface composition / %			
	CHx (285 eV)	**C-OR (286.7 eV)**	**O-C-O (288.1 eV)**	**COOR (290 eV)**
Natural hemp fibre	79.6	14.3	4.7	1.4
Hemp fibres after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	37.9	28.5	24.9	8.6
Natural sisal fibre	60.1	26.8	7.1	6.0
Acetone treated sisal fibre after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	34.6	43.5	16.5	5.4

Table 4: Surface energy dispersive component and acid-base numbers of bacterial cellulose, natural fibres and modified fibres.

Sample	γ_s^d (mJ/m²)	K_A	K_B
Bacterial cellulose	61.0	0.11	0.41
Natural hemp fibre	40.7	0.11	0.12
Hemp fibres after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	39.9	0.10	0.24
Natural sisal fibre	38.4	0.11	0.07
Sisal fibre acetone extracted	32.4	0.08	0.28
Acetone treated sisal fibre after sterilisation at 121ºC for 20 min in medium modified with bacterial cellulose	35.1	0.08	0.34
NaOH pretreated hemp fibre modified with bacterial cellulose	61.0	0.15	0.20
NaOH pretreated sisal fibre modified with bacterial cellulose	61.9	0.17	0.10

Table 5. Interfacial shear strength (IFSS) between fibres and renewable matrices.

Treatment	**IFSS to CAB / MPa**	**IFSS to PLLA^{50}/ MPa**
Natural sisal fibre (Sisal-N)	1.02 ± 0.06	12.1 ± 0.5
Sisal fibre modified with bacterial cellulose (Sisal-NBC)	1.49 ± 0.03	14.6 ± 1.2
Acetone treated sisal fibre	-	9.5 ± 0.7
Acetone treated sisal fibre modified with bacterial cellulose	-	Internal Failure
Natural hemp fibre (Hemp-N)	0.76 ± 0.06	-
Hemp fibre modified with bacterial cellulose (Hemp-NBC)	1.83 ± 0.12	-

Scheme captions

Scheme 1: Structure of lignin and cellulose monomers.

Figure captions

Figure 1: SEM micrograph showing the gap at the interface between natural hemp fibre and poly-L-lactic acid (PLLA) polymer matrix

Figure 2: Cassette designed to contain natural fibre mat (or loose fibres) and drawing of its position in the fermentor vessel.

Figure 3: Photographs of sisal fibres before and after 2 days of the bacterial culture.

Figure 4: SEM micrographs of hemp fibre surfaces; (a) Natural hemp fibre; (b) Hemp fibre after bacterial cellulose modification.

Figure 5: SEM micrographs of sisal fibre surfaces; (a) Natural sisal fibre; (b) Sisal fibre to which bacterial cellulose was attached; (c) Acetone-treated sisal fibre after bacterial cellulose modification.

Figure 6: (a) Photograph of the cassette covered with bacterial cellulose pellicle after fermentation; (b) Photograph of the fibres inside the cassette; (c) Photograph of sisal fibre bonded with bacterial cellulose network.

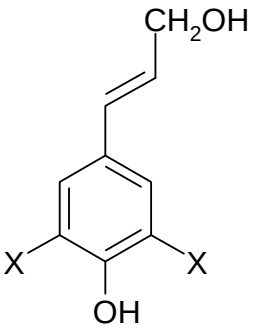
Figure 7: SEM micrograph of sisal fibre surface after cultured in fermentor, showing its partially coverage of bacterial cellulose.

Figure 8: Deconvoluted C 1s XP spectra of hemp and sisal fibres before and after bacterial cellulose modification.

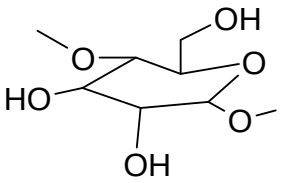
Figure 9: SEM micrographs of: (a) bacterial cellulose-modified sisal; (b) acetone-treated and bacterial cellulose modified sisal fibres, and the corresponding CAB matrix cavities after single fibre pullout testing.

Figure 10: Single fibre pullout results for hemp and sisal fibres in CAB matrix; (□) Natural sisal fibre (Sisal-N); (O) Sisal fibre modified with bacterial cellulose (Sisal-NBC); (◊) Natural hemp fibre (Hemp-N); (Δ) Hemp fibre modified with bacterial cellulose (Hemp-NBC).

Scheme 1



Lignin monomer
(X = H, OMe)



Cellulose monomer

Figure 1

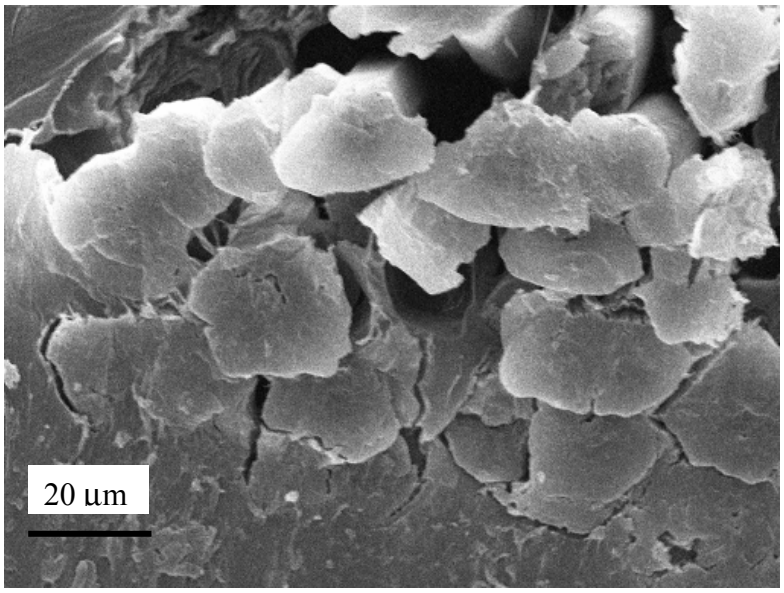
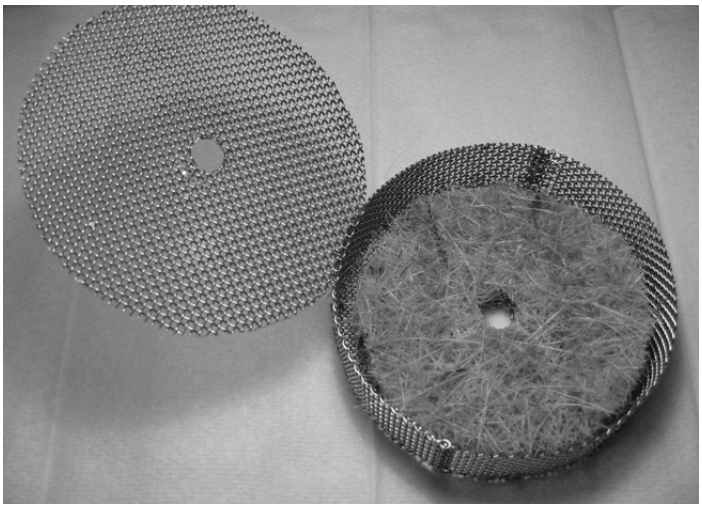


Figure 2



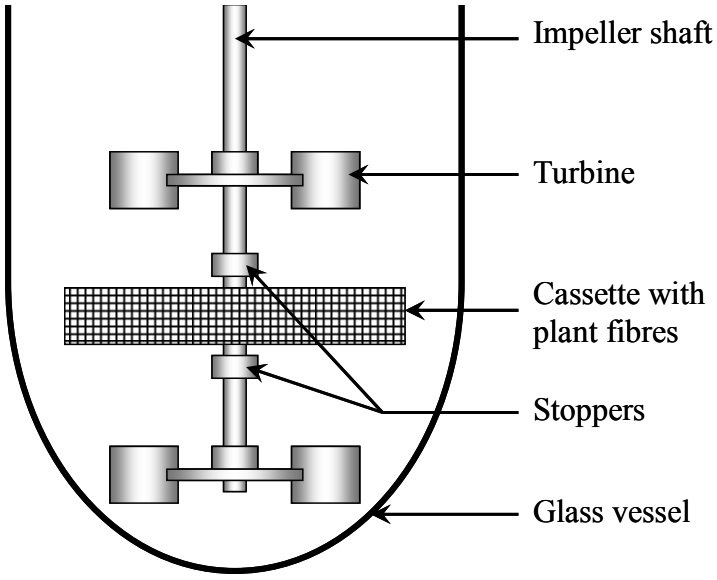

Impeller shaft
Turbine
Cassette with plant fibres
Stoppers
Glass vessel

Figure 3

Figure 4

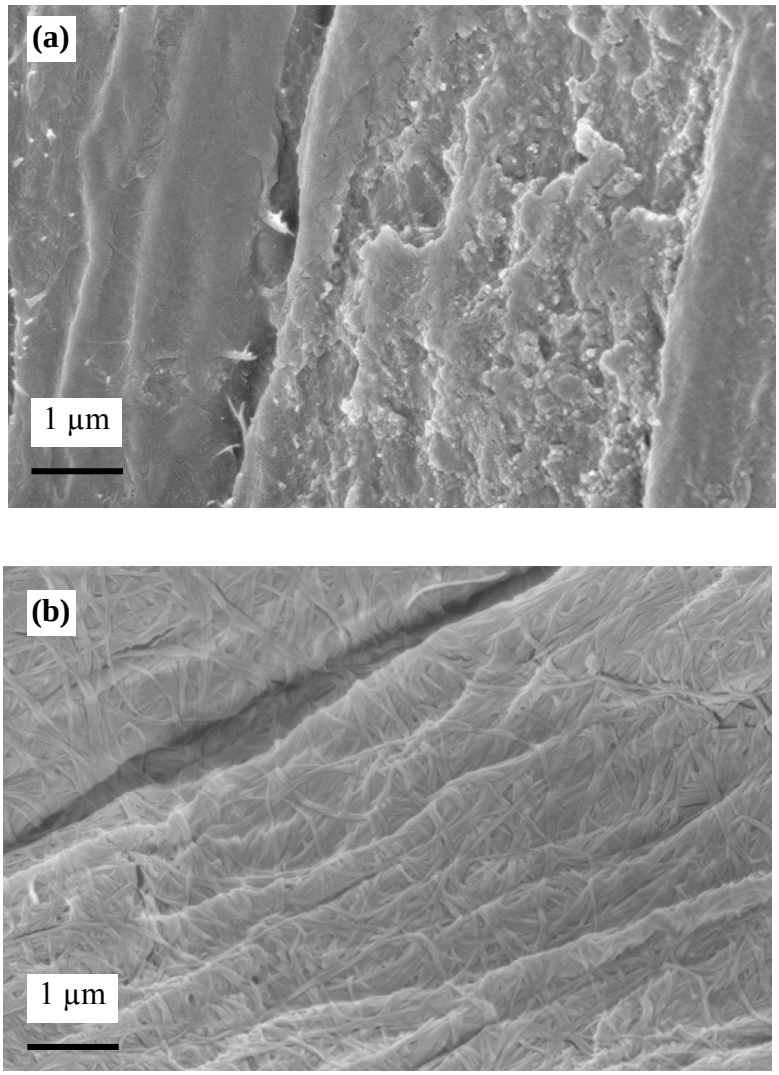


Figure 5

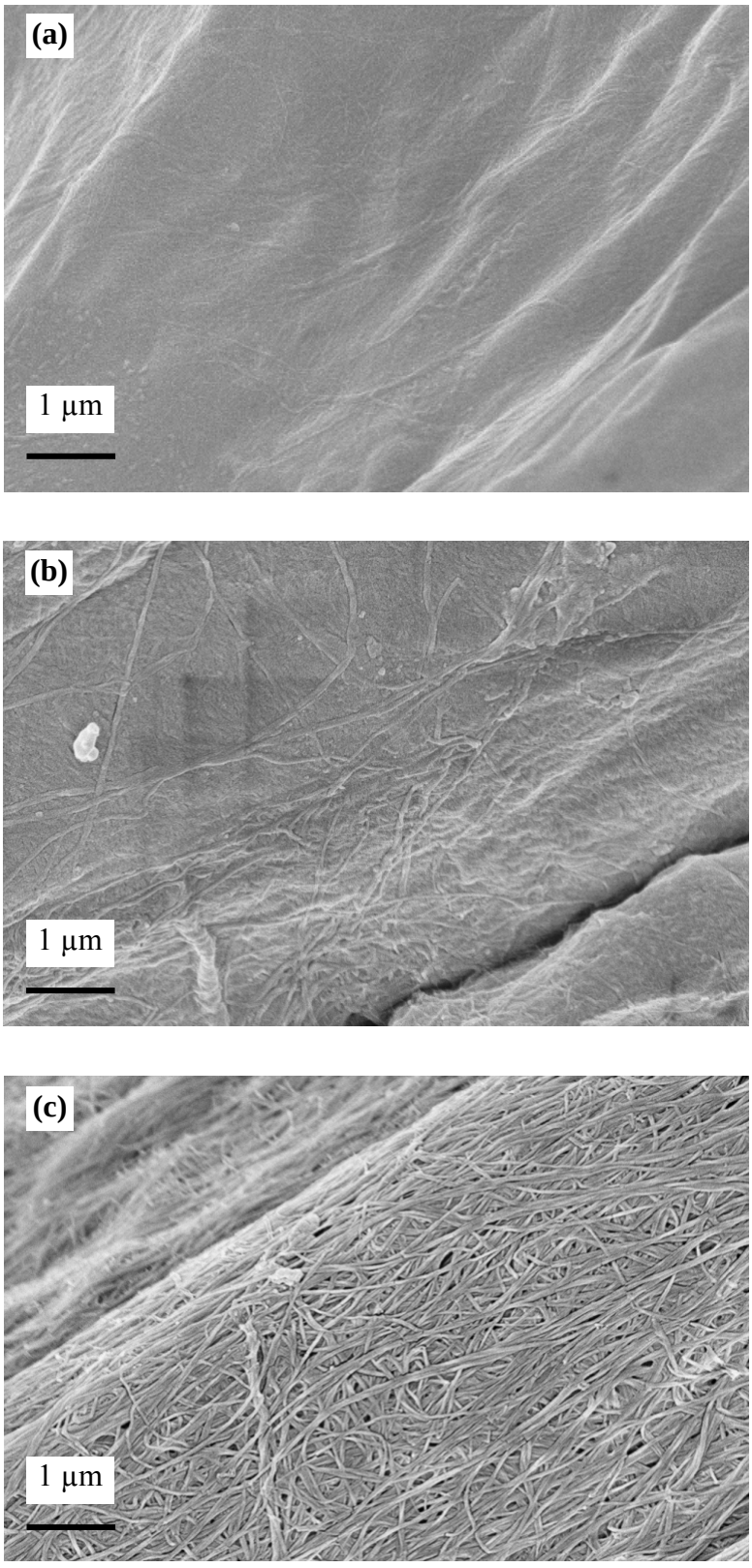
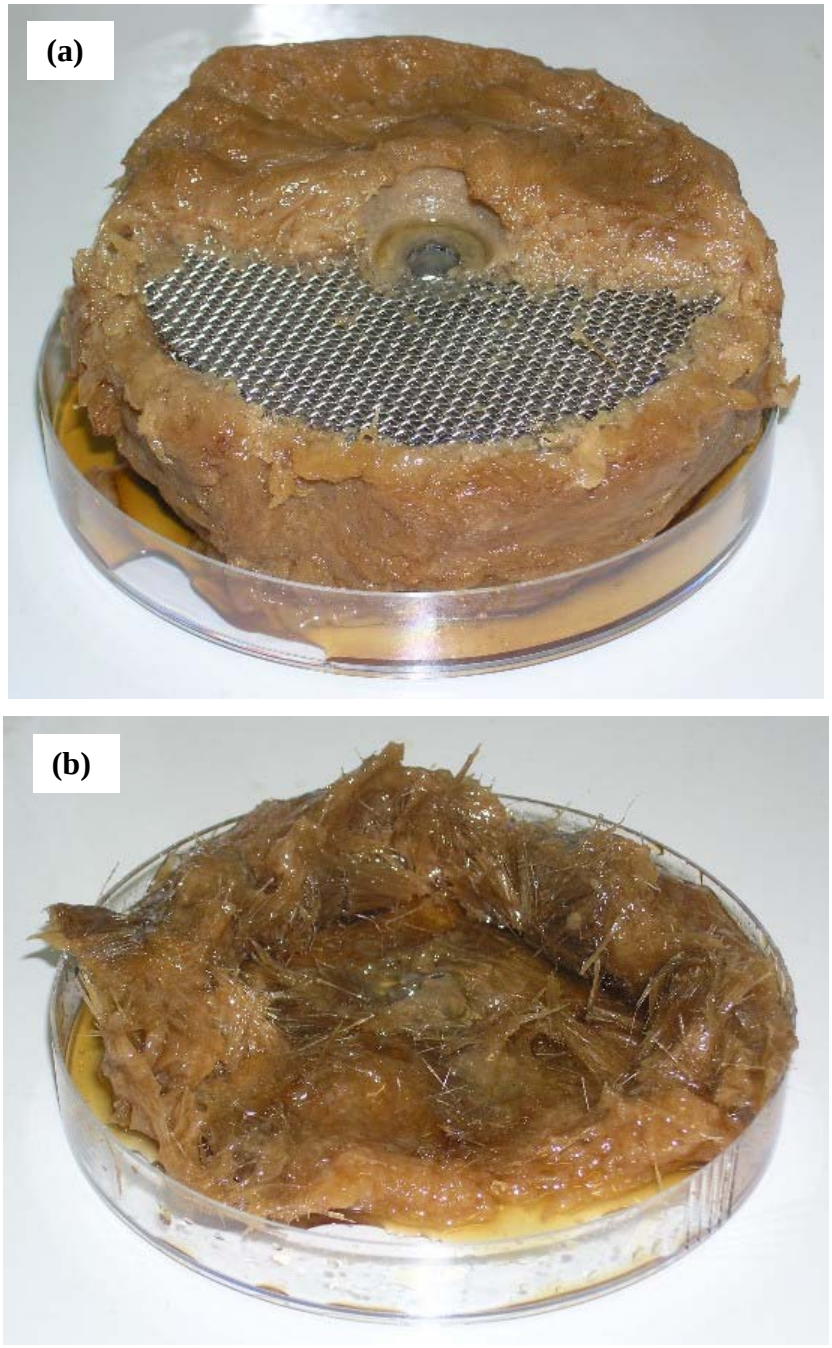


Figure 6




(c)

Figure 7

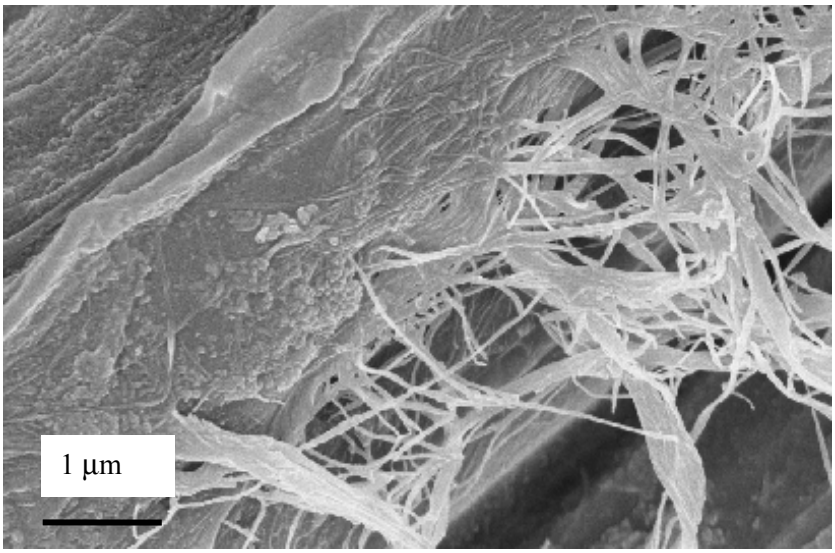


Figure 8

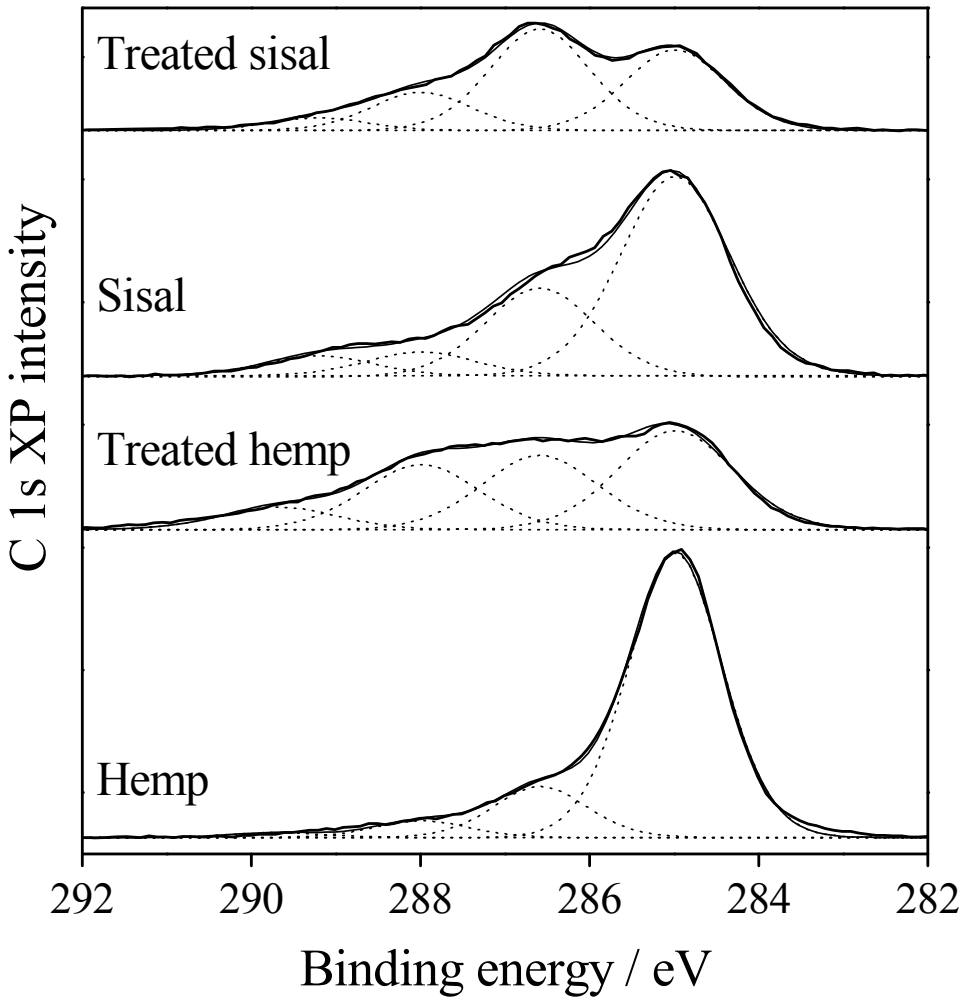

Treated sisal
Sisal
Treated hemp
Hemp
C 1s XP intensity
292
290
288
286
284
282
Binding energy / eV

Figure 9

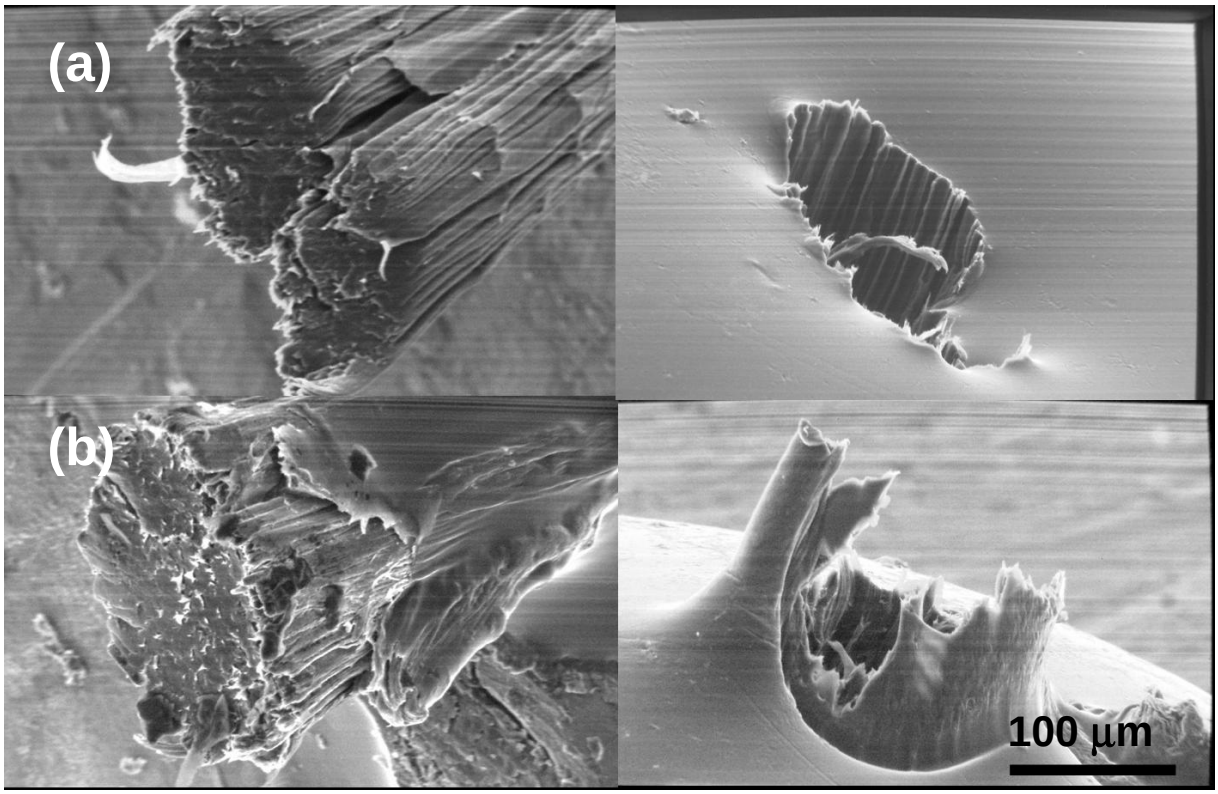
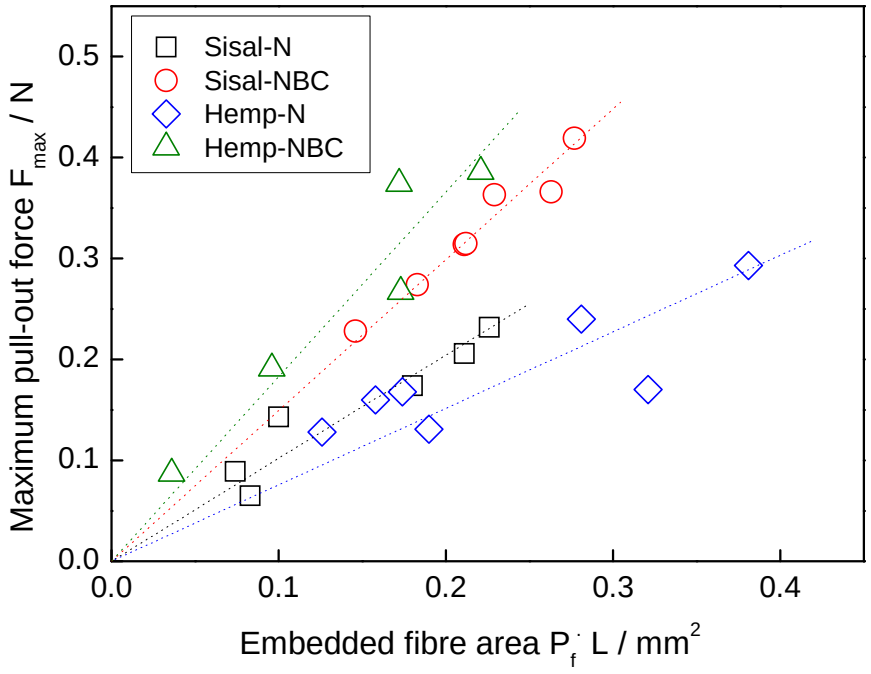


Figure 10



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Manuscript Title: Surface modification of natural fibres using bacteria: Depositing bacterial cellulose onto natural fibres to create hierarchical fibre reinforced nanocomposites

Authors: Marion Pommet, Julasak Juntaro, Jerry Y.Y. Heng, Athanasios Mantalaris, Adam F. Lee, Karen Wilson, Gerhard Kalinka, Milo Shaffer and Alexander Bismarck*