Sustainable optically transparent composites based on epoxidized soy-bean oil (ESO) matrix and high contents of bacterial cellulose (BC)

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Abstract Production of transparent composites from totally renewable resources with extraordinary potential for different applications can be made possible using cellulose. Composites of epoxidized soybean oil (ESO)/bacterial cellulose (BC) nanofibers have been prepared with high fiber content. Due to the nano-order scale network-like structure of BC nanofibers, composite films present high transparency even at high BC content. Transparency of films has been analyzed by UV–visible spectroscopy observing that only 15% of matrix transmittance is lost in the nanocomposites. ESO/BC composites show better mechanical properties with increasing BC content. Composites combine high stiffness and good ductility due to the incorporation of BC network structure in ESO matrix.

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Introduction

Bacterial cellulose (BC) nanofibers are a form of cellulose produced by bacteria such as *Gluconacetobacter xylinum*. Despite their identical chemical compositions, the structure and mechanical properties of BC differ from those of plant cellulose. BC nanofibers are several nanometers wide with very high aspect ratio. They present excellent mechanical properties including tensile strength and modulus, high water-holding capacity, high moldability, crystallinity, and biocompatibility, that make them excellent reinforcement for polymeric matrices (Borzani and Desouza 1995; Chao et al. 2000; Putra et al. 2008; Yan et al. 2008).

Recently novel composites with high plant or bacterial cellulose nanofiber content and high transparency have been developed (Iwamoto et al. 2005; Nakagaito and Yano 2008). Due to the size effect, cellulose nanofiber-reinforced composites can retain the transparency of the resin even at high fiber contents. Moreover, since BC nanofibers are aggregates of semicrystalline extended cellulose chains forming a rigid network-like structure, they also can contribute to a significant improvement on thermal and mechanical properties. However, only a few

works have been developed related to composites of bacterial cellulose with matrices from renewable resources (Tang and Liu 2008). As well known, cellulose is incompatible with hydrophobic polymeric matrices, which results in poor interfacial adhesion. This fact, along with nanofiber dispersability problems makes their potential reinforcing efficiency is not used in the composites. In order to avoid these problems, surface modification of nanofibers, such as acetylation, becomes necessary. Acetylation treatment esterifies the surface hydroxyl groups of cellulose nanofibers. Grafting of acetate groups reduces the hydrogen bonding ability of the initial hydroxyl groups which increases the probability of interaction between hydrophobic resin and cellulose nanofibers.

The polymeric matrix used to make BC composites must satisfy some conditions such as low initial viscosity, easily controlled gelation times, and good adhesion and wettabilities to the cellulose. Epoxy and acrylic resins satisfy these requirements and had been used to make nanocomposites based on BC. Despite that epoxy matrices have good final properties, the increasing concern about the environmental protection have rise to the search of epoxidized substitutes derived from renewable resources. In this sense, bio-based polymer products derived from annually renewable agricultural and biomass feedstock have become important in material science and applications as raw materials from petroleum are limited to Earth′s reserves and also because environmental concern is increasing. Triglyceride plant oils represent a major class of such renewable resources. Soybean oil (SBO) is the most readily available and one of the lowest cost vegetable oils in the world. In the past decade much effort has been dedicated to producing SBO-based polymeric materials. Epoxidized soybean oil (ESO) is manufactured by the epoxidation of the double bonds of the SBO triglycerides with hydrogen peroxide, either in acetic acid or in formic acid, and it is industrially available in large volumes at a reasonable cost (Boquillon and Fringant 2000; Jin and Park 2008; Takahashi et al. 2008).

In this study, preparation of ESO/BC composites, both of the components from renewable resources, with high BC content has been produced by immersion process. Their transparency, morphology mechanical properties have been characterized. Lab made BC nanofibers have been functionalized using acetylation route for improving both nanofiber dispersion and adhesion at the nanofiber/matrix interface. The nanocomposites showed optically transparency and excellent mechanical properties.

Materials and methods

Materials

Epoxidized soybean oil was kindly supplied by Hebron S. A. and cured with hexahydrophthalic anhydride (HHPA) from Fluka using 1-methyl imidazol from Fluka as catalyst. Tetrahydrofuran (THF) was used as solvent. Acetic acid (98 wt%), perchloric acid (0.1 N), toluene and acetic anhydride from Panreac were used for acetylation of BC nanofibers.

BC films

Bacterial cellulose (BC) was obtained from a *Gluconacetobacter xylinum* pellicle (made in the laboratory) incubated for 13 days at 28 °C in a static culture, as described elsewhere (Retegi et al. 2010). BC pellicles with a thickness of 0.5–0.7 cm were boiled in 5 wt% KOH solution for 60 min at 120 °C in order to remove cells, and then thoroughly washed under running water for 2 days to obtain pure BC nanofibers.

For BC films fabrication, BC nanofibers were suspended in water (0.5 wt%) and stirred for 48 h. Then suspension was vacuum filtered using nylon membrane filter (0.2 μm mesh) producing 25 mm diameter mats that were oven dried at 70 °C for 48 h between glass plates. Mats were compression moulded at 100 MPa at 70 °C in order to increase stiffness of the material, thus obtaining 30–40 μm thickness BC nanofiber films.

BC nanofiber surface was modified by acetylation route (Kim et al. 2002) in order to improve the compatibility with ESO resin. A solution of 20 mL toluene and 20 mL acetic acid was prepared and then 0.2 mL H_3PO_4 and 2 mL acetic anhydride were added and stirred until homogeneous solution was obtained. After immersion of BC films in the solution, treatment was performed for 1 h at room temperature. Finally samples were removed from the solution and washed with methanol to eliminate residual impurities, and then compression-molded at 70 °C. Finally samples were oven dried under vacuum at 60 °C to avoid water sorption problems. These samples are identified as BCace.

Composite preparation

BCace films (with a IR acetyl index of 0.77, measured following Hurtubise method 1962), were immersed in THF containing ESO resin, hardener and catalyst for 2 h in vacuum. The weight ratio of each of the components of the system was 122.5 g ESO, 76 g hardener, 2.58 g catalyst and the ratio (resin, hardener and catalyst):solvent was 1:3. Thereafter, samples were immersed in THF for 5 and 15 min to remove the excess of surface resin obtaining ESO/25BCace and ESO/75BCace composites with 25 and 75 wt% BC nanofibers contents, respectively. Then, vacuum treatment at 120 °C during 5 min was carried out to remove bubbles. Finally samples were cured at 120 °C for 4 h and post-cured for 1 h at 180 °C in Teflon molds to prevent sticking. The amount of BC nanofibers in composites was calculated from the weight difference between the weight of the sample (BCace film) previous immersion cycle and the final sample weight. Samples of each system showed a maximum deviation around 5 wt%.

Characterization

Atomic force microscopy

Atomic force microscopy (AFM), NanoScope III controller with a MultiMode head, from Digital Instruments was used in order to characterize the morphology of BC nanofibers in ESO/BCace composites. For every sample, analysis was performed in Tapping Mode (TM) in air under moderate conditions for recording height and phase images. Silicon cantilevers with a resonance frequency of about 200–400 kHz and a spring constant of 12–103 N/m were used for imaging.

UV–visible spectroscopy

Transparency of the films was evaluated in a UV–visible spectrometer Jasco UV-630. In order to assure the homogeneity of the sample, five tests of each film were performed in cured ESO and ESO/BCace films, and also in BCace films from 200 to 1,100 nm at 25 °C.

Mechanical tests

Tensile tests were performed in a Minimat miniature mechanical tester machine using a 200 N load cell. The crosshead rate used was 1 mm/min and distance between grips was fixed at 22 mm, following ASTM D 1708 standard. At least five specimens were tested for each set of films, being the average value reported.

Results and discussion

Transparency of composites

In Fig. 1, photographs of the composite components and ESO/BCace composites are shown. At first glance, the transparency of cured ESO film is remarkably higher than that of mostly opaque white coloured BCace nanofiber film. When mixing these two materials, the cured ESO/BCace composite retained the transparency of ESO matrix even at high BCace content (ESO/75BCace), which indicates the uniform dispersion of BC nanofibers at fiber content as high as 75 wt%. Several procedures were used to prepare composites with unmodified cellulose varying ESO/THF ratio, temperature, hardener …, but in all cases the obtained composites were opaque. Though not shown, cured ESO/BC films were hardly transparent,
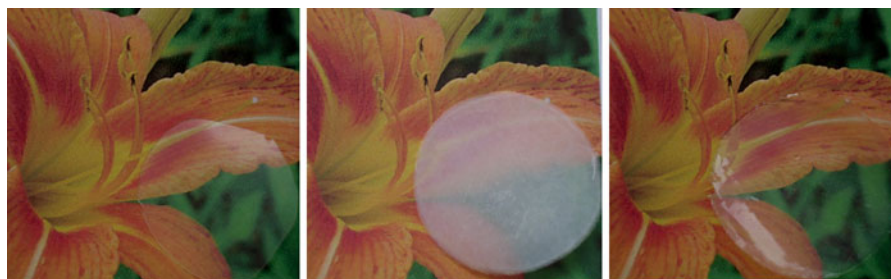

Fig. 1 Photographs of cured ESO matrix, BCace and cured ESO/75BCace composite films
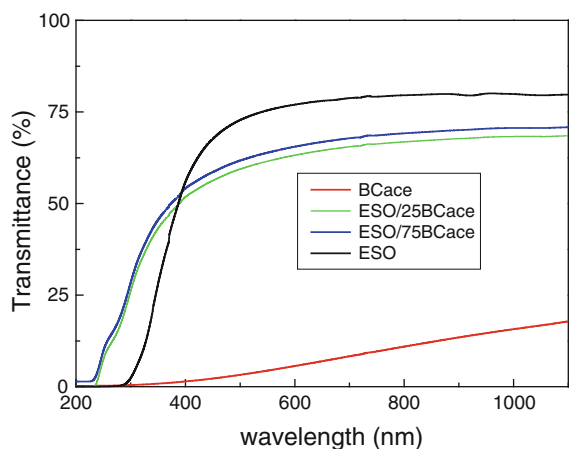
Fig. 2 Light transmittance curves of cured ESO matrix, ESO/BCace composites and BCace films

even for low BC content. This fact, added to the transparency of composites containing BCace nanofibers, indicates a better matching of their refraction index with that for the matrix and increased interfacial interactions between the modified nanofibers and epoxy matrix.

Figure 2 shows results of UV–vis tests for cured ESO matrix, BCace and cured ESO/BCace composite films. According to the curves presented, cured ESO is a polymer with a great degree of transparency in all analyzed wavenumber range (75% at 600 nm), that makes it suitable for any optical application. In the case of BCace film, the highest transmittance value of 18% occurs around 1,000 nm, which indicates it presents a high degree of translucency. Finally, cured ESO/75BCace composite film present high degree of transparency, as only a 15% loss is observed when compared with the cured ESO film. These results are in accordance with those obtained by Tang and Liu (2008) in transparency tests for poly(vinyl alcohol)/bacterial cellulose nanofiber composites. Shimazaki et al. (2007) also ensured high degree of transparency in epoxy matrix/bacterial cellulose nanofibers based composites. The high volumetric fraction of BCace nanofibers that can be achieved on these composites, without loosing the transparency of ESO matrix, is linked to the three-dimensional network-like structure of bacterial cellulose nanofibers (Iwamoto et al. 2005). It is well-known that, when adding a reinforcement to a matrix in ordinary composites, a decrease in transparency occurs due to the scattering of light radiation produced by both reinforcement size and the

difference in refractive index between each of the phases (Novak 1993). Additionally, though the refractive indices are very similar (until the third decimal), any change in temperature can cause translucency of the composite (Tang and Liu 2008). Usually, translucency evolutes to opacity when the goal is to improve the mechanical properties of composite, and the volume fraction of reinforcement is increased (Nogi et al. 2005). In composites obtained with BCace nanofibers, these obstacles can be avoided. First, the structure of the BCace network consists of fibers with diameters ten times smaller than the wavelength of visible light. Thus, in some works the "size effect" presented by BC nanofiber-based composites has been defined to explain why even using high percentages of BC nanofibers (up to 75 wt%) the scattering of light can be avoided, thus achieving transparent composites (Yano et al. 2005). Moreover, compared with other reinforcements, the use of BC nanofibers opens a range of possibilities for matrices of different refractive indexes. In Kyoto University, only 20% loss of transparency was observed in composites of phenol/formaldehyde matrix (index of refraction = 1.483), several acrylic (1.396–1.488) and epoxy systems (1.522) reinforced with up to 75 wt% BC [16]. In this case, the used ESO resin has a refractive index of 1.470–1.475 and BC nanofibers have a refractive index of 1.618 in the fiber direction and 1.544 perpendicular to fiber direction. Despite that, all the obtained composites showed high degree of transparency. Therefore, it is clear that BC nanofiber-based composites open new possibilities for the use of polymers of different refractive indices to prepare high performance transparent materials based on renewable resources, such as that used in this work.

Morphology of the samples

Figure 3 shows AFM image of ESO/BCace composite with 75 wt% BCace nanofiber content. As seen, there are not great differences with the image obtained for BCace films in Fig. 4, as a network-like structure of uniformly distributed cellulose nanofibers can be observed in both cases. The reason for this similarity may be based on the high percentage of cellulose present in the composite, as ESO matrix is placed between the cellulose nanofibers microgaps. Yano et al. (2005) also did not observe great differences in AFM images from bacterial cellulose-based epoxy
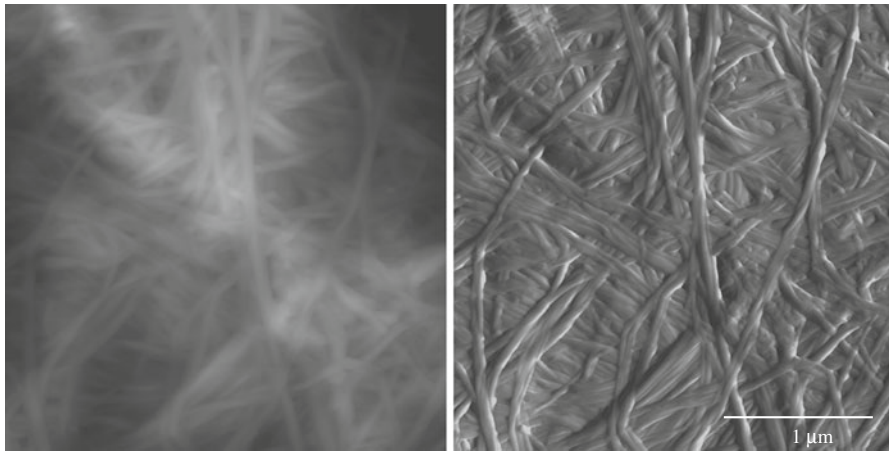
Fig. 3 3 μm × 3 μm AFM image of ESO/75BCace composite film: *left/right* heigth/phase

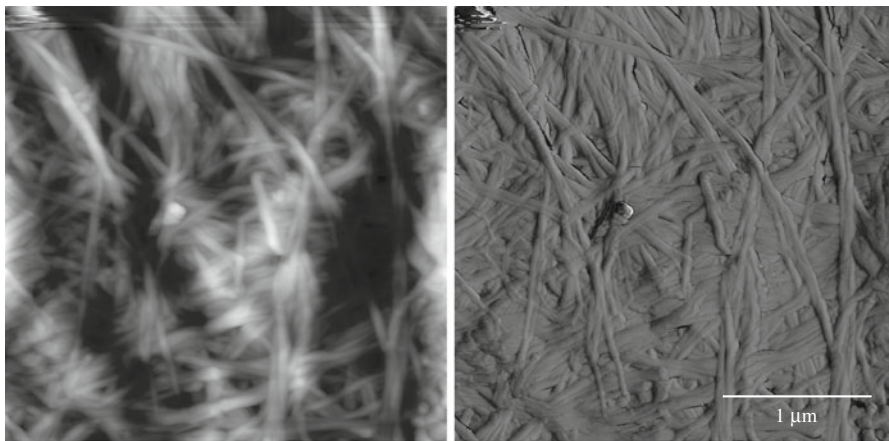**Fig. 4** 3 μm × 3 μm AFM image of BCace film: *left/right* heigth/phase

matrix composites and the one taken for neat bacterial cellulose nanofiber film. Therefore, according to AFM image of ESO/75BCace composite, during the dipping process of BC nanofiber film, the resin was poured in the gaps of the BCace nanofiber films without distancing nanofibers and, thus, retaining the integrity of the film. Therefore, composites can take advantage of the mechanical stability of these films.

Mechanical behaviour

In Fig. 5 stress–strain curves of composites and each of their components are shown, whereas data obtained from these tests can also be found in Table 1. As expected, when increasing BCace nanofiber content in ESO/BCace composites, higher stiffness and strength were obtained. This behaviour can be related to the three-dimensional network structure of bacterial cellulose as it is uniformly distributed in the composite, thus giving high stiffness to the material. Composite elastic modulus shows a nearly linear relationship with BCace nanofiber content, as higher values of this property are obtained for increasing nanofiber content. Nakagaito and Yano (2008) observed a linear relationship of elastic modulus in fenol-formaldehyde/microfibrillated cellulose composites for increasing nanofiber contents. However, increasing cellulose content over 40 wt%, they found deviations to the linear relationship which was associated to the reinforcing saturation effect. In fact, when BCace nanofiber content is high, some difficulties were found to fill the resin in the cellulose nanofibers microgaps,
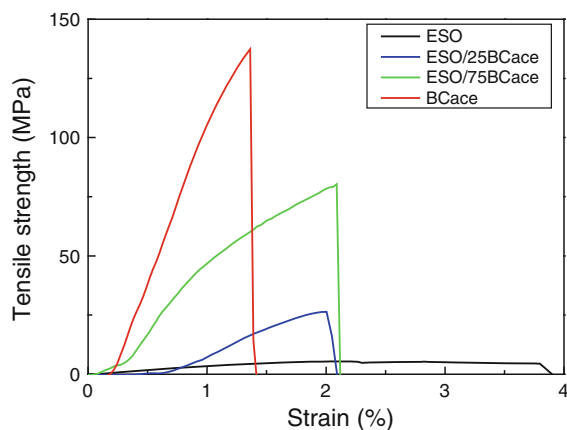
Fig. 5 Stress-strain curves of cured ESO matrix, and ESO/BCace composites, and BCace films

Table 1 Mechanical properties of cured ESO matrix, and ESO/BCace composites and BCace film

Sample	Elastic modulus (GPa)	Tensile strength (MPa)	Strain at break (%)
ESO	0.45 ± 0.1	5.5 ± 0.4	3.6 ± 0.5
ESO/25BCace	2.8 ± 0.4	25 ± 0.4	2.1 ± 0.5
ESO/75BCace	5.9 ± 0.5	81 ± 0.7	2.1 ± 0.4
BCace	9.2 ± 0.7	135 ± 8.0	1.8 ± 0.1

giving rise to materials with some homogeneity problems. Also in our case, following linear relationship, elastic modulus of ESO/25BCace and ESO/75BCace nanocomposites should be certainly higher, that means work should be done in processing of these nanocomposites to enhance still more their mechanical behaviour.

Likewise, tensile strength also displayed the same trend, as nanocomposite strength increased with BCace nanofiber content. The reason for this behaviour is based on the three-dimensional network of cellulose nanofibers in the composite and the resin penetration into the voids, thus increasing the density of interaction between BCace nanofibers and ESO system and preventing crack spread that would carry the samples to a catastrophic rupture. Nakagaito and Yano (2005) observed the same trend in microfibrillated cellulose reinforced composites and associated it to the interconnected cellulose microfibrils surfaces.

With regard to strain at break of cured ESO film, it is slightly higher than that of BCace nanofibers film. The maximum strain of ESO resin depends on the used

hardener and curing conditions as ESO resins with brittle to rubber-like behaviour can be found in the literature (Sharma and Kundu 2006). In this case, the addition of BCace nanofibers decreased strain at break of composites. However, taking into account the tensile strength and maximum strain values obtained, composites show great ability to absorb energy before breaking.

Summarizing, BC nanofibers offer promising characteristics as a reinforcement material for optically multifunctional composite materials. Moreover, the renewable resource origin of both BC nanofibers and ESO resin make these composites sustainable and very attractive for the development of high performance materials.

Conclusions

In this work, transparent composites based on high content of acetylated bacterial cellulose nanofibers synthesized from *Gluconacetobacter xylinum* bacteria and ESO epoxy system have been produced. Composites show excellent mechanical properties regarding elastic modulus and strength. This behaviour is related to the three-dimensional network-like structure formed by dimensionally identical and uniformly distributed cellulose nanofibers that promoted interfibrillar bonding during bacterial cellulose films fabrication. Moreover, the resin penetration into the microgaps between nanofiber gives cohesion, stiffness and deformability to the composites. In addition, despite the high nanofiber content (up to 75 wt%), transparency of the cured matrix was retained in the composites due to the "size effect" of cellulose nanofiber. Stiffness, high tensile strength and transparency of these materials make them useful for a wide range of industrial applications. Moreover, taking into account the renewable resource origin of both BC and ESO resin, these materials can constitute an important basis for the development of "green materials" in composite material science.

References

Boquillon N, Fringant C (2000) Polymer networks derived from curing of epoxidised linseed oil: influence of different catalysts and anhydride hardeners. Polymer 41:8603–8613

Borzani W, Desouza SJ (1995) Mechanism of the film thickness increasing during the bacterial production of cellulose on non-agitated liquid-media. Biotechnol Lett 17:1271–1272

Chao Y, Ishida T, Sugano Y, Shoda M (2000) Bacterial cellulose production by *Acetobacter xylinum* in a 50-L internal-loop airlift reactor. Biotechnol Bioeng 68:345–352

Hurtubise FG (1962) The analytical and structural aspects of the IR spectroscopy of cellulose acetate. TAPPI 45:460–465

Iwamoto S, Nakagaito AN, Yano H, Nogi M (2005) Optically transparent composites reinforced with plant fiber-based nanofibers. Appl Phys A Mater Sci Process 81:1109–1112

Jin FJ, Park SJ (2008) Impact-strength improvement of epoxy resins reinforced with a biodegradable polymer. Mater Sci Eng A 478:402–405

Kim DY, Nishiyama Y, Kuga S (2002) Surface acetylation of bacterial cellulose. Cellulose 9:361–367

Nakagaito AN, Yano H (2005) Novel high-strength biocomposites based on microfibrillated cellulose having nano-order-unit web-like network structure. Appl Phys A Mater Sci Process 80:155–159

Nakagaito AN, Yano H (2008) The effect of fiber content on the mechanical and thermal expansion properties of biocomposites based on microfibrillated cellulose. Cellulose 15:555–559

Nogi M, Handa K, Nakagaito AN, Yano H (2005) Property enhancement of optically transparent bionanofiber composites by acetylation. Appl Phys Lett 87:243110

Novak BM (1993) Hybrid nanocomposite materials-between inorganic glasses and organic polymers. Adv Mater 5:422–433

Putra A, Kakugo A, Furukawa H, Gong JP, Osada Y (2008) Tubular bacterial cellulose gel with oriented fibrils on the curved surface. Polymer 49:1885–1891

Retegi A, Gabilondo N, Peña C, Zuluaga R, Castro C, Gañan P, de la Caba K, Mondragon I (2010) Bacterial cellulose films with controlled microstructure-mechanical property relationship. Cellulose 17:661–669

Sharma V, Kundu PP (2006) Addition of polymers from natural oils. A review. Progress Polym Sci 31:983–1008

Shimazaki Y, Miyazaki Y, Takezawa Y, Nogi M, Abe K, Ifuku S, Yano H (2007) Excellent thermal conductivity of transparent cellulose nanofiber/epoxy resin nanocomposites. Biomacromolecules 8:2976–2978

Takahashi T, Hirayama KI, Teramoto N, Shibata M (2008) Biocomposites composed of epoxidized soybean oil cured with terpene-based acid anhydride and cellulose fibers. J Appl Polym Sci 108:1596–1602

Tang C, Liu H (2008) Cellulose nanofiber reinforced poly(vinyl alcohol) composite film with high visible light transmittance. Comp Part A 39:1638–1643

Yan Z, Chen S, Wang H, Wang B, Jiang J (2008) Biosynthesis of bacterial cellulose/multi-walled carbon nanotubes in agitated culture. Carbohydr Polym 74:659–665

Yano H, Sugiyama J, Nakagaito AN, Nogi M, Matsuura T, Hikita M, Handa K (2005) Optically transparent composites reinforced with networks of bacterial nanofibers. Adv Mater 17:153–155