Carbohydrate Polymers 116 (2015) 173–181

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Characterization of purified bacterial cellulose focused on its use on paper restoration

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ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form 19 February 2014
Accepted 26 March 2014
Available online 3 April 2014

Keywords:
Bacterial cellulose
Characterization
Purification
Paper restoration
Aging

ABSTRACT

Bacterial cellulose (BC) synthesized by *Gluconacetobacter sucrofermentans* CECT 7291 seems to be a good option for the restoration of degraded paper. In this work BC layers are cultivated and purified by two different methods: an alkaline treatment when the culture media contains ethanol and a thermal treatment if the media is free from ethanol. The main goal of these tests was the characterization of BC layers measured in terms of tear and burst indexes, optical properties, SEM, X-ray diffraction, FTIR, degree of polymerization, static and dynamic contact angles, and mercury intrusion porosimetry. The BC layers were also evaluated in the same terms after an aging treatment. Results showed that BC has got high crystallinity index, low internal porosity, good mechanical properties and high stability over time, especially when purified by the alkaline treatment. These features make BC an adequate candidate for degraded paper reinforcement.

1. Introduction

Cellulose is the most abundant polysaccharide in nature. It is synthesized by plants, some animals and a large number of microorganisms (Bielecki, Krystynowicz, Turkiewicz, & Kalinowska, 2005). *Gluconacetobacter sucrofermentans* (previously *Gluconacetobacter xylinus*) is a gram-negative bacterium, strictly aerobic, that produces cellulose from different carbon sources (Ramana, Tomar, & Singh, 2000) at temperatures ranging between 25 and 30 °C and pHs varying from 3 to 7 (Bielecki et al., 2005; Iguchi, Yamanaka, & Budhiono, 2000). Cellulose is a primary metabolic product of these bacteria and plays an important role in keeping them in contact with the oxygen-rich surface, protecting the cells from ultraviolet light and retaining moisture (Klemm, Shuman, Udhardt, & Marsch, 2001).

Bacterial cellulose (BC) synthesis occurs as a multi-step series of chemical reactions beginning with the incorporation of monomeric glucose into β-1,4-glucan chains (Jonas & Farah, 1998) that aggregate into subfibrils, which then are extruded through pores arranged on the cell surface. Approximately 10–100 subfibrils entangle together to form crystalline microfibrils of around 3.5 nm in diameter (Jonas & Farah, 1998). Subsequently, these microfibrils are gathered in bundles, which group to form ribbons (Yamanaka, Ishihara, & Sugiyama, 2000). Ribbons intertwine with ribbons from other bacterial cells, forming a two-dimensional layer. Finally, parallel layers interact with one another by hydrogen bonds and Van der Waals forces, generating a gelatinous suspension or pellicle, in the liquid medium (Brett, 2000). Water absorbed by the BC layers can be removed, leaving chemical groups available for new hydrogen bonds between adjacent cellulose chains, a process that increases crystallinity (Colvin & Leppard, 1977).

BC is chemically identical to that produced by plants, but it is organized in a different macrostructure. BC shows higher crystallinity (Nakagaito, Nogi, & Yano, 2010), mechanical strength and purity (Castro et al., 2011), because it is free from lignin, hemicellulose and other biopolymers and extractives typical of the vegetal cell wall. On account of its unique properties, BC has multiple applications in fields as diverse as paper, textile and food industries, also as a biomaterial for manufacturing cosmetics, artificial skin, artificial blood vessels or high fidelity speakers (Chawla, Bajaj, Survase, & Singhal, 2009).

BC's properties and possible uses are highly dependent on its structure. Therefore, this paper reports on the morphology and structure of optimal BC pellicles produced by *G. sucrofermentans*

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http://dx.doi.org/10.1016/j.carbpol.2014.03.064
0144-8617/

CECT 7291, in order to increase the knowledge on its future application in paper restoration. Culture media were previously optimized for this application, selecting fructose and CSL-yeast extract as carbon and nitrogen sources, respectively (Santos, Carbajo, & Villar, 2013).

Two methods of paper restoration with BC could be used: (1) separate production of BC layers to be subsequently applied over the document that is to be restored; and (2) direct generation of BC on the surface of the document. Although the addition of 1% ethanol has been reported to improve BC production (Yunoki, Osada, Kono, & Takai, 2004), this results in a significantly lower pH (Santos et al., 2013), which is a major disadvantage for method (2) because the document may be damaged, but not for method (1) because the layer is washed before its application on the paper getting an adequate pH. So, in order to study this possibility also, here, half the BC samples were cultivated on fructose and CSL-yeast extract adding 1% ethanol to the culture medium.

Regardless of the presence or absence of ethanol, a purification step has to be added to remove bacteria and the culture medium from the BC before its future application to paper. Previously, several assays for killing bacteria were tested and two options were selected: If BC is going to be applied as described in method (1), the purification was achieved with an alkaline treatment, a standard procedure to remove bacteria and the culture medium. However, this alkaline treatment could adversely affect the document if BC is going to be generated directly on its surface, that is to say, in method (2). In this case, to prevent damage to the document, a gentler thermal treatment was used. Afterwards, an aging process was applied in both cases to evaluate the BC performance over time, with characterization being conducted before and after aging.

BC samples were characterized in terms of their mechanical properties (tear and burst strength), optical properties, surface properties (static contact angles), porosity (Hg intrusion porosimetry), crystallinity (SEM, X-ray diffraction), and FTIR response. Mechanical, optical and surface properties, as well as porosity, are parameters of interest in papermaking and they will determine the use and durability of BC in paper restoration. Other properties, such as crystallinity and viscosity, relate to the structure and integrity of BC, while the FTIR response has been used to determine the presence of non-cellulosic substances in the samples.

2. Materials and methods

2.1. Microorganism

G. sucrofermentans CECT 7291 was obtained from the Spanish Type Culture Collection (CECT). For maintenance, it was subcultured periodically in HS medium (Hestrin & Schramm, 1954). *G. sucrofermentans* was grown in HS solid medium placed in Petri dishes for 6 days, in order to obtain the suspension of bacterial cells to be used in subsequent experiments. These plates were inoculated into 500 ml Erlenmeyer flasks containing 100 ml of liquid HS medium and cultivated in static conditions for 4 days. Subsequently, the pellicles formed were cut in small pieces (approx. 1 cm × 1 cm) in sterile conditions and shaken with the liquid medium at 700 rpm for 30 min. The suspension obtained was filtered through gauze, centrifuged at 4000 rpm for 10 min and, after removing the supernatant, the pellet was washed with Ringer's solution (NaCl, 2.5 g/L; KCl, 0.105 g/L; $CaCl_2{\cdot}2H_2O$, 0.120 g/L; and $NaHCO_3$, 0.05 g/L). This solution was centrifuged again in the same conditions and the pellets were re-suspended in a small volume of Ringer's. The optical density of the solution was adjusted to 0.59–0.64 (McFarland standards 3–4), with a wavelength of 600 nm diluting with Ringer's solution. Aliquot parts of 250 μL of this final solution were inoculated into 100 mL of medium.

2.2. Production of cellulose layers

Culture media used for bacterial cellulose production were: (1) a modified HS medium (fructose, 20 g/L; yeast extract, 5 g/L; corn steep liquor, 5 g/L; Na_2HPO_4, 2.7 g/L; and citric acid, 1.15 g/L); and (2) medium of equal composition plus 1% ethanol. In all cases 100 mL of liquid medium were added to 150 mm Petri dishes, which went on to be inoculated with the suspension described above and cultivated at 30 °C under static conditions. Cellulose layers were analyzed after 7 days of cultivation. At least 5 different layers were tested for each property.

2.3. Purification of cellulose layers

The cellulose pellicles were washed with distilled water. Pellicles grown in ethanol containing medium were incubated at 90 °C in 1% NaOH for 60 min (Ethanol-BC-Alkaline Treatment: E-BC-AT). Pellicles produced in ethanol free medium were kept at 65 °C for 24 h (no Ethanol-BC-Thermal Treatment: nE-BC-TT). Pellicles were exhaustively washed under a continuous distilled water flow after both treatments. To measure the isolated effect of purification on BC, non-treated samples were only exposed to washing with continuous distilled water (Ethanol-BC: E-BC and no Ethanol-BC: nE-BC). Finally, cellulose films were dried by filtering through medium porosity filter paper in a Buchner funnel plus air drying.

2.4. Aging of cellulose layers

BC layers were submitted to an accelerated aging process at 80 °C and 65% relative humidity, for 144 h; according to ISO 5630-3:1996. Prior to their characterization, aged and not aged dry films were conditioned under standard conditions (23 °C and 50% relative humidity), according to ISO 187:1990.

2.5. Fourier transform infrared spectroscopy (FTIR)

A Jasco FTIR-4600 spectrophotometer, equipped with an accessory single reflection diamond, was used to perform the analysis. Resolution of the spectrophotometer was set at 2 cm^{-1}, and 100 scans were obtained in the 4000–650 cm^{-1} spectral region.

2.6. X-ray diffraction (XRD)

Bacterial cellulose crystallinity was studied by XRD using a multipurpose PAN analytical diffractometer, model X'Pert MPD. This diffractometer is equipped with a copper X-ray tube and two goniometers with vertical configuration th-2th and Bragg–Brentano optic. One of the goniometers has a multipurpose sample support, which can hold large samples as heavy as 1 kg and measuring up to 10 cm × 10 cm × 10 cm. The supporting platform of the second goniometer is a sample spinner fitted with an automatic sampler with 21 positions. The diffractometer is used in phase analysis and, in this study, the angular range between $2\theta = 5–40^\circ$ was studied.

The crystallinity indexes for the BC samples have been calculated in each case using the equation (Retegi et al., 2010):

$$\text{Crystallinity}\,(\%) = \frac{I_{200} - I_{2\theta=18^\circ}}{I_{200}}.$$

2.7. Viscosity

The limiting viscosity number has been determined after dissolving BC in cupriethylenediamine, according to ISO 5351:2010. The only variation in our study was that BC was dissolved directly

in diluted cupriethylenediamine solution, without previously disintegrating it in water.

2.8. Mercury intrusion porosimetry

Pore structure of the cellulose layers was assessed by the mercury intrusion test, using a 9320 Porosizer from Micromeritics. This technique consists in forcing mercury into the sample pores at increasing pressures (from 3.5 MPa to 227,000 MPa). The output data is the volume of mercury intruded into the sample as a function of the pressure applied, which is inversely proportional to the size of the pores. To provide a curve of the pore size distribution, the differential volumes of mercury imbibed at a given pressure were plotted versus the mean pore diameter. The volume of cellulose pores within a specific diameter range is proportional to the area under this curve.

2.9. Static and dynamic contact angle

The static contact angle measurements were performed in a Data Physics Instrument OCA 15 plus, running on SCA 20/21 software and using the sessile drop method. The images were taken by a CCD camera immediately after the drop landed on the surface of the cellulose layer. The corresponding contact angle is calculated after fitting the drop contour line numerically, using the Young–Laplace method. In this study, 20 drop tests were conducted with distilled water drops of 2 μL.

Dynamic wettability was assessed monitoring the change over time of the contact angle with water. The same automated contact angle tester was used. For each sample, five videos recorded the evolution of the drop during 60 s, taking one frame per second, applying droplets of 2 μL. In order to quantify differences in the evolution of the contact angles (α), the wettability rate for each sample was calculated as follows:

$$\text{Wettability rate} = \frac{(\alpha_t - \alpha_5)}{(t-5)}$$

where t is 60 s. As droplets only become stable after the first 5 s, this time interval is subtracted.

2.10. Scanning electron microscopy

The morphology of the BC layers was studied by SEM microscopy using a JEOL JSM 6335F at 1 kV (with maximum resolution of 5 nm) to avoid energetic degradation of the samples during SEM observation. Such samples were metalized with gold during 3 min and stored for 16–18 h at 50 °C in a vacuum stove (20 mmHg) before proceeding with the SEM observations. This latter treatment led to total dryness of the samples.

2.11. Mechanical and optical properties

The following mechanical properties were determined: Grammage (ISO 536:2012), burst strength (ISO 2759:2001) and tear strength (ISO 1974:2012). A Color Touch reflectometer (Datacolor®) was used to assess the optical properties: ISO brightness (ISO 2470-1:2009), opacity (ISO 2471:2008) and yellowness index (SCAN-G 5:03). Yellowness is defined as the attribute by which an object color is judged to depart from a preferred white toward yellow.

3. Results and discussion

A previous study has assessed the efficiency of the purification methods described above (thermal and alkaline) by inoculating HS Petri dishes with pieces of treated and untreated BC. *G. sucrofermentans* only grew in dishes that had untreated BC, which proved that these methods kill the bacteria efficiently.

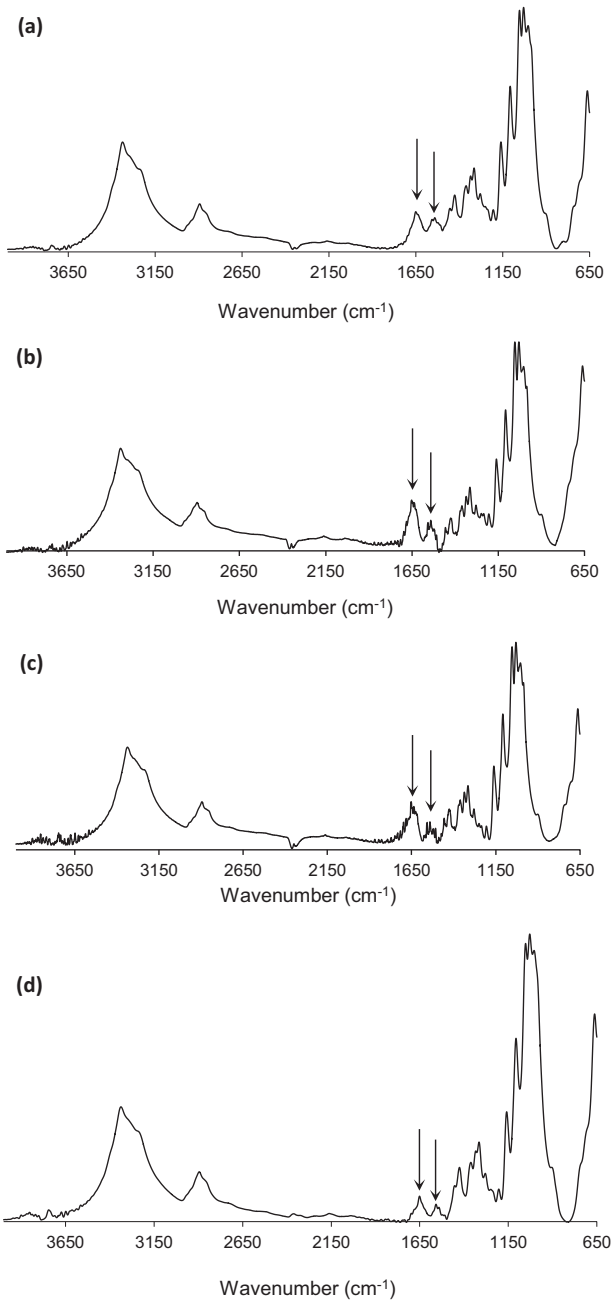

Fig. 1. FTIR spectroscopy of non-aged cellulose layers. (a) nE-BC, (b) E-BC, (c) nE-BC-TT, (d) E-BC-AT.

3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy enables to evaluate the efficiency of the purification method and the structure of the cellulose layers by observing the resulting absorption bands. Fig. 1 shows the typical bands of cellulose in FTIR spectra (C—O bonds at 1055, C—H bonds at 2900, and O—H bonds at 3340 cm^{-1}). Sugiyama, Persson, and Chanzy (1991) have reported that signals near 750 and 3240 indicate the existence of type Iα crystalline cellulose, and signals near 710 and 3270 are indicative of type Iβ (Fig. 1); therefore, both polymorphisms are present in BC.

According to Duvey and Saxena (2002), the two peaking signals approximately at 1536 and 1640 cm^{-1} correspond to the amide bond and can be associated with proteins and residual biomass, that is, biological impurities likely originated from the bacteria or compounds in the culture medium that may have remained attached to the cellulose layers. It can be observed (Fig. 1a and b) that cellulose layers generated in the presence of 1% ethanol (E-BC) result in higher bands than those from samples produced without ethanol (nE-BC). The reason behind this finding may be that ethanol would promote the development of bacteria and result in more byproducts, such as organic acids (Li et al., 2012).

The amount of impurities does not change when the treatment used to kill the bacteria was the thermal treatment; nE-BC-TT compared with nE-BC (Fig. 1a and c). However, with the alkaline purification treatment (E-BC-AT) the two peaking bands were much lower than those of E-BC (Fig. 1b and d). Hence, it can be inferred that even though a treatment at 65 °C kills bacteria, it does not eradicate them. In contrast, the NaOH treatment does eradicate the bacteria by cell lysis (Barud et al., 2008) and chemical bond breakage.

When the aging process is applied, the FTIR spectrum of the BC layers (data not shown) shows no significant differences with results obtained before the aging process. Thus, it can be concluded that the aging process does not disrupt the structure of BC nor alters the efficiency of the purification methods. This stability supports the feasibility of using BC in paper restoration.

3.2. X-ray diffraction (XRD)

Fig. 2 shows the results from XRD for the four samples of non-aged (Fig. 2A) and aged bacterial cellulose (Fig. 2B). Each of these has two diffraction dominant peaks, the first one located between 13° and 16° and the second one located between 21° and 25°. Each peak presents both the crystalline phase Iα and Iβ. Barud et al. (2008) investigated the composite membranes of BC and suggested that the first peak represents the projection of the planes (1 0 0) of fraction Iα and (1 1 0 and 0 1 0) of fraction Iβ, and the second peak represents the projection of the planes (1 1 0) Iα and (2 0 0) Iβ.

The crystallinity indexes (Table 1) are higher than those obtained with vegetal cellulose pulps. Typical values are 74.9% for *Eucalyptus grandis* pulp and 75.5% for *Pinus taeda* pulp (Poletto, Pistor, Zeni, & Zattera, 2011).

Cheng, Catchmark, and Demirci (2009) obtained similar crystallinity indexes using the same strain to study the influence of additives in BC production. It can be observed that the most crystalline layers are produced with the alkaline treatment (E-BC-AT), probably because NaOH can solubilize amorphous cellulose and because the alkaline treatment removes proteins and nucleic acids of the bacterial cells (George, Ramana, Sabapathy, Jagannath, & Bawa, 2005), which increases the crystallinity of the material. The crystallinity increases with aging, which may be to the result of metabolites getting leached by the combined effect of temperature and humidity.

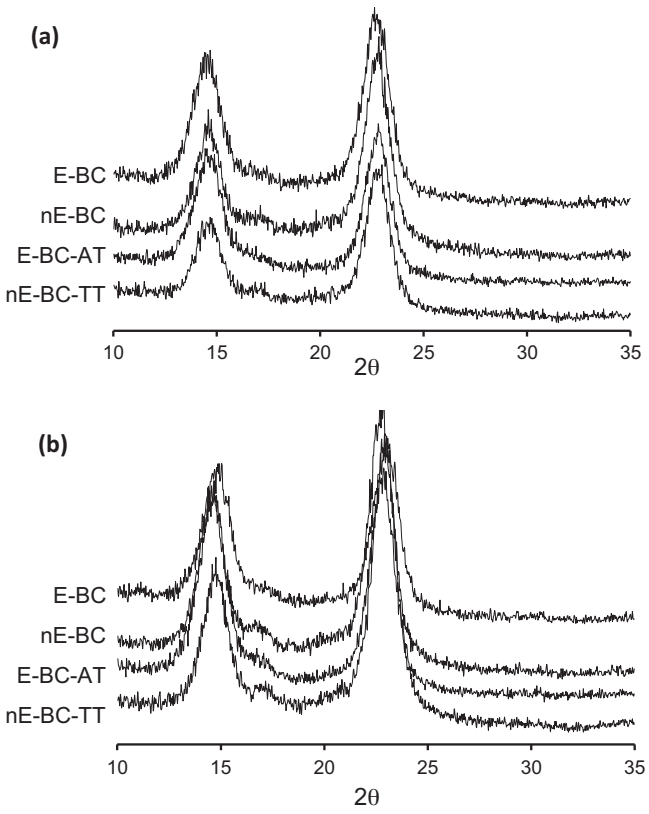

Fig. 2. X-ray diffraction patterns of non-aged (a) and aged (b) BC samples.

3.3. Viscosity

Intrinsic viscosity gives an estimation of the average degree of polymerization of the cellulose, and indicates the relative integrity of cellulose fibers. Viscosity of vegetal cellulose pulp for paper production usually varies from 550 to 950 mL/g (Lapierre, Bouchard, & Berry, 2009). As can be observed in Table 1, viscosity of BC is, in most cases, higher than these values.

The thermal treatment, that facilitates the removal of certain metabolites, increases viscosity. The alkaline treatment promotes a bigger viscosity increment secondary to the marked purification (George et al., 2005) and the elimination of low molecular weight cellulose (Jahan, Rawsan, Nasima Chowdhury, & Al-Maruf, 2008). The increment in viscosity produced when the purification method is the alkaline treatment correlates with the crystallinity.

Table 1
Structural properties of BC samples.

	Crystallinity index (%)	Viscosity (mL/g)	Total porosity (%)	Static contact angle (°)	Wettability rate (°/s)
No Ethanol-BC					
Non-aged	83.4	820	66.2	51.5	0.29
Aged	88.6	900	52.4	65.0	0.18
No Ethanol-BC-Thermal Treatment					
Non-aged	83.8	1050	53.3	84.9	0.29
Aged	89.8	830	49.4	76.4	0.15
Ethanol-BC					
Non-aged	86.8	950	56.0	72.3	0.28
Aged	87.2	1030	56.6	76.2	0.16
Ethanol-BC-Alkaline Treatment					
Non-aged	87.7	1700	61.9	41.3	0.21
Aged	90.0	1220	59.3	42.7	0.14

Aging of cellulose layers previously treated causes the degradation of the cellulose chains. However, the slight increase in viscosity observed in non-treated layers after the aging process may be related to the leaching of low molecular weight metabolites due to the high temperature and humidity conditions of aging process. This removal would compensate the effect of cellulose chain degradation which was mentioned before. In treated layers this low molecular weight compounds have been previously removed during the treatments.

3.4. Mercury intrusion porosimetry

Fig. 3 shows curves of pore size distribution for the four samples, before and after the aging process. This technique has been used and broadly discussed for paper sample characterization, but not for BC layers. Several authors have described two types of paper porosity: (a) surface porosity or pores larger than 10 μm mainly placed on the surface of the sheet, and (b) internal porosity or pores with diameters of less than 10 μm (Chinga-Carrasco, Axelsson, Eriksen, & Svensson, 2008; Moura, Ferreira, & Figueiredo, 2005). The present study has used the same criteria in the analysis of bacterial cellulose layers. As it is shown in Fig. 3, the greatest differences are observed in the range of diameters >10 μm. In non-aged samples, nE-BC layers have got the greatest surface porosity, while E-BC layers have a smaller pore fraction.

Table 1 shows the total porosity of the BC layers. The thermal treatment causes a considerable reduction of the total pore volume when compared with nE-BC, while the alkaline treatment results in an increment in the total porosity. A likely explanation for this finding is that NaOH kills and promotes the removal of bacteria, as previously shown by FTIR, and as a consequence, releases the space occupied by the microorganisms and, therefore, increases the pore volume. The high temperature of the aging process causes a diminution of the pore volume, which is consistent with the decrement in porosity caused by the thermal treatment. At high temperatures, water is partially removed from the pores and new bonds are formed between adjacent cellulose chains, a phenomenon known as hornification (Fernandes Diniz, Gil, & Castro, 2004).

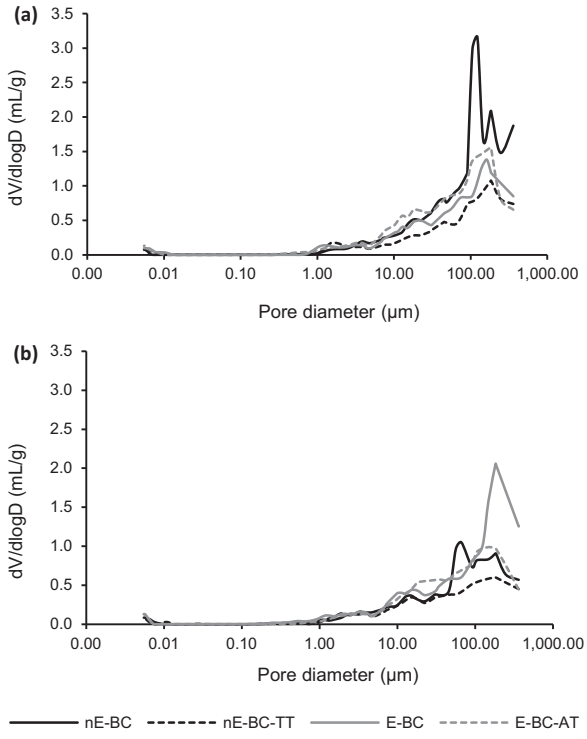

Fig. 3. Pore size distribution for non-aged (a) and aged (b) BC samples.

3.5. Static and dynamic contact angles

To know how the BC layers surfaces behave against liquids, static and dynamic contact angles are determined. These measurements

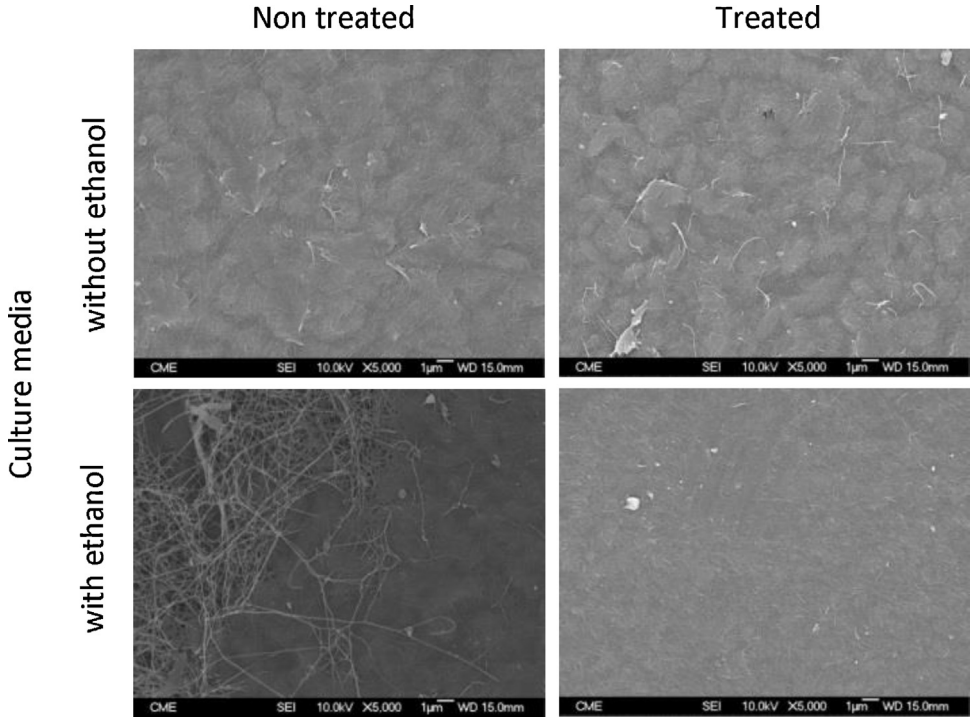

Fig. 4. SEM images of the surfaces of BC layers.

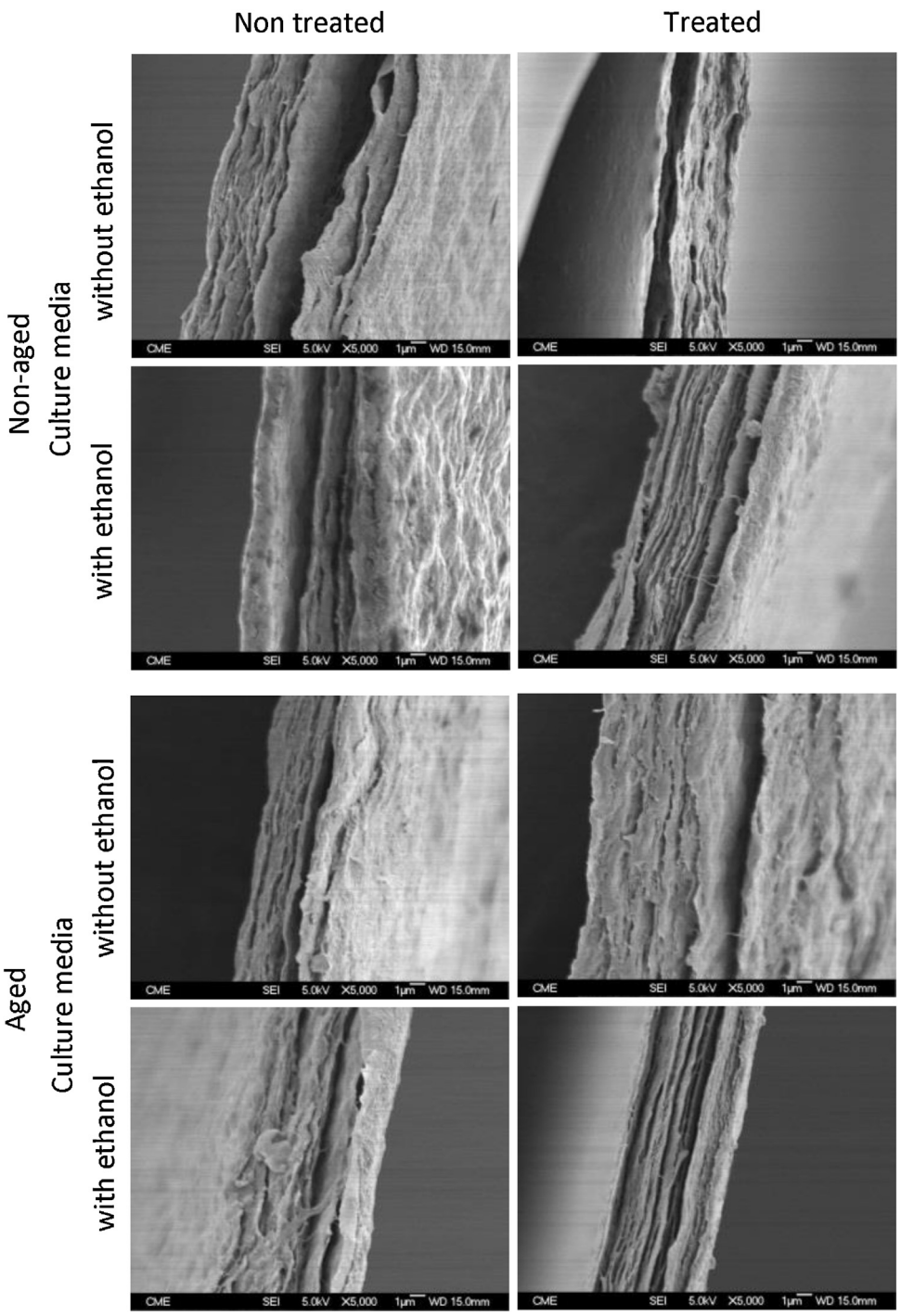

Fig. 5. SEM images of the transversal sections of BC layers.

are widely used to characterize paper printing process, but they have never been used to characterize bacterial cellulose.

The static contact angles with water (α) for the eight samples are listed in Table 1. When comparing the layers without treatments, it becomes evident that angles are greater in the E-BC layers than in nE-BC layers, which might be the result of ethanol contributing to generate a cellulose layer with a certain surface hydrophobicity.

The comparison of the layers without treatments with their respective treated layers shows that the nE-BC-TT sample gets a higher α value than the nE-BC sample, which may a result of the purification process removing certain polar metabolites and causing hydrophobicity. However, this trend is not consistent with what is observed in E-BC and E-BC-AT layers. Cellulose swelling is a well documented effect of NaOH, and it is responsible for the α decrease (Young, 1994). The results observed with the aged samples clearly show that aging did not change especially the outcome significantly, and this was especially the case of the alkaline treatment, which seems to render very stable cellulose layers.

The wettability rate (°/s) is estimated from the dynamic contact angle. All measured rates were rather low: the droplets were hardly absorbed or spread on the surfaces of the BC layers after sixty seconds. The wettability rates get even lower after the aging

Table 2
Optical properties of BC samples.

	Yellowness (%)	Opacity (%)	Brightness (%)
No Ethanol-BC			
Non-aged	21.4	18.6	45.6
Aged	40.2	21.1	34.8
No Ethanol-BC-Thermal Treatment			
Non-aged	28.4	21.8	41.2
Aged	43.3	26.1	32.6
Ethanol-BC			
Non-aged	26.8	24.5	42.8
Aged	35.0	25.1	38.5
Ethanol-BC-Alkaline Treatment			
Non-aged	−3.5	16.0	66.1
Aged	27.7	20.3	43.0

process, and this may be due to hornification resulting in a decrease in porosity with aging, as capillarity phenomena play an important role in wettability on long-time scales. This is an interesting point to bear in mind because preventing water absorption could be positive to restored papers.

3.6. Scanning electron microscopy

The superficial morphology of BC dried layers is shown in the SEM micrographs (Fig. 4), where the ultrafine network of cellulose nanofibres can be observed. It can be clearly noticed that the thermal treatment does not remove the bacterial cells from the layer. In contrast, no bacterial cells are observed after the alkaline treatment. The SEM micrographs of the aged samples (not shown) allowed us to conclude that aging produces no change on the surface.

Fig. 5 shows the SEM micrographs corresponding to the transversal sections of the sheets, before and after aging. The decrement of porosity in the nE-BC-TT samples may be observed. In all cases, aging leads to compaction of the bacterial cellulose sheet, which is consistent with the decrement of porosity and increment of crystallinity index previously described.

High temperatures also decrease the distance between layers in every BC sheet, which is consistent with the results of the thermal treatment and with the assessment of total porosity (Table 1) which have been discussed before.

3.7. Mechanical and optical properties

As it is shown in Fig. 6, with the exception of alkali-treated layers (E-BC-AT), the grammage (Fig. 6a) is similar across all cases. The alkaline treatment lyses cell walls, and this facilitates the removal of the bacteria, as well as short chain cellulose, metabolites and other impurities, which leads to a decrease in basis weight of about 36%. This is consistent with the values reported by George et al. (2005), as they found a decrement in the grammage of about 15–20% when the treating with 0.2 N NaOH. Slight grammage changes observed with aging may be attributed to the samples reaching moisture equilibrium with different degrees of humidity (Hotle, Considine, Wald, Rowlands, & Turner, 2008).

As it can be observed in Fig. 6b and c, BC has high burst strength and acceptable tear strength to use it in paper restoration. In the non-treated samples, there were no significant differences between burst indexes before aging; likewise, the tear index was quite uniform across non-treated non-aged samples. Purification produced by the alkaline treatment results in a significant increase in the tear strength (Fig. 6c) that is consistent with the remarkable increase in viscosity described above, but which also causes a slight decrease of burst strength (Fig. 6b). Aging process does not necessarily produce a detriment in mechanical properties, even in some cases can improve them due to cellulose crosslinking (Zervos, 2010). In our case, aging process causes no significant differences in the tear index, just a decrease in the E-BC-AT samples. But there is an important decrease in the burst index in nE-BC-TT and E-BC samples, which nevertheless remain higher than values measured in vegetal cellulose paper.

Optical properties are shown in Table 2. When comparing the two non-treated samples, the E-BC layers have got higher

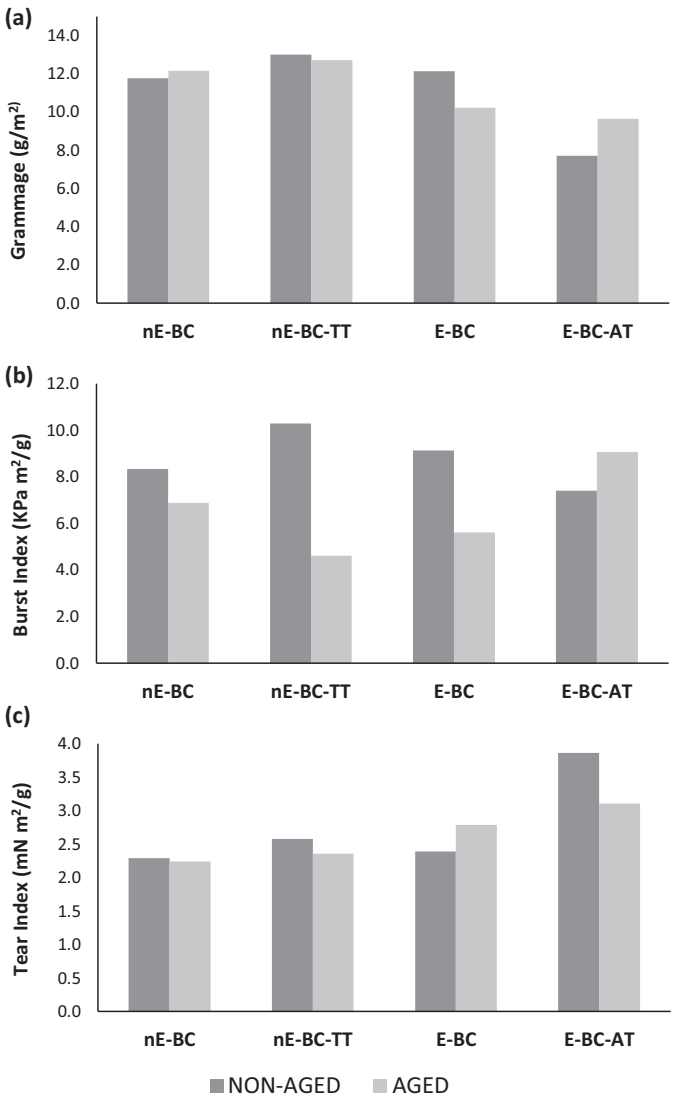

Fig. 6. Mechanical properties of BC samples. (a) Grammage, (b) Burst Index, (c) Tear Index.

yellowness and opacity, and slightly lower brightness. These findings can be attributed to the presence of a higher number of bacterial cells and, also, other metabolites caused by the presence of ethanol in the culture medium (Li et al., 2012).

The thermal treatment results in an increase in yellowness and a decrease in brightness which is common when cellulose undergoes a thermal aging process (Ardelean, Bobu, Niculescu, & Groza, 2011). Alkaline treatment causes a significant decrease in yellowness and an important increment in brightness, consistent with the above discussion as this treatment produces a significant purification of the cellulose layers. Also, this treatment causes a significant decrease in opacity, which, in addition to the elimination of numerous metabolites and cells, may be associated to the significant decrease in basis weight.

The aging process results in a common outcome across all samples examined: an increase in yellowness and a decrease in brightness (as expected), and an increase in opacity consistent with the compaction of the layers observed in the micrographs, as well as with the decrease of porosity.

4. Conclusions

This study deals with the characterization of BC layers focused on its future use on paper restoration. The way to obtain and purify BC layers depends on their possible enforcement modes: for an intended synthesis on the surface of the document, BC layers are produced in a culture medium free from ethanol and purified with a thermal treatment. For an ex situ production of BC layers to be subsequently applied over the document, they are produced in a culture medium with 1% ethanol and purified with an alkaline treatment. Significant differences were found between these methods. In general it can be concluded that bacterial cellulose has high crystallinity index, low internal porosity, and good mechanical properties.

The alkaline treatment promotes an increment in viscosity, crystallinity and purity in the cellulose layers, and eliminates bacterial cells. Remarkable mechanical and optical properties are observed in the cellulose so treated. In contrast, the thermal treatment is less aggressive, rendering layers of similar quality to non-treated ones, although a slight purification occurs that leads to a small improvement in viscosity and mechanical properties.

The BC stability over time was tested by means of an aging process. In view of the results, it may be concluded that aging causes an increment in crystallinity. It also causes a diminution of pore volume. These findings are consistent with those of the thermal treatment. The alkaline treatment provides great stability over time in terms of contact angles, porosity and crystallinity. After the aging process, BC has nearly the same tear index and lower burst index, which nevertheless remains higher than values measured in vegetal cellulose paper.

In both cases (alkaline or thermal treatment) BC may improve the physical properties of the damaged paper and, also, provides stability over time. In thermal treated BC layers, the presence of residual bacterial cells may be an inconvenient and will be tested in future works.

This study suggests that BC could be a promising material for restoration of paper documents because its characteristics and its high stability over time indicate that it can contribute to the reinforcement of degraded paper.

Acknowledgments

The authors wish to thank A. Sánchez Hernampérez, from the National Library of Spain for his invaluable help. The Spanish Ministry of Science and Innovation funding via Project CTQ 2010-17702 is also acknowledged.

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