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1 **Bacterial cellulose for increasing barrier**  
2 **properties of paper products**

3

4 Amanda Fillat<sup>a</sup> (0000-0003-0453-1178), Josefina Martínez<sup>a</sup> (0000-0002-2411-8188),  
5 Cristina Valls<sup>b</sup> (0000-0003-2307-1779), Oriol Cusola<sup>b</sup> (0000-0002-1407-8285), M.  
6 Blanca Roncero<sup>b\*</sup> (0000-0002-2694-2368), Teresa Vidal<sup>b</sup> (0000-0001-6269-4114),  
7 Susana V. Valenzuela<sup>a</sup> (0000-0002-1684-9514), Pilar Diaz<sup>a</sup> (0000-0003-4008-0669),  
8 F.I. Javier Pastor<sup>a\*</sup> (0000-0003-0326-2527)

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10 *<sup>a</sup>Department of Genetics, Microbiology and Statistics. Faculty of Biology. University of*  
11 *Barcelona. Av. Diagonal 643, 08028 Barcelona, Spain*

12 *<sup>b</sup>CELBIOTECH\_Paper Engineering Research Group. Universitat Politècnica de*  
13 *Catalunya. BarcelonaTech. 08222 – Terrassa. Spain*

14

15 \*Authors for correspondence: F.I.J Pastor, E-mail: fpastor@ub.edu . Tel.: +34-93-4034626. Fax: +34-93-  
16 4034629 and M. Blanca Roncero, E-mail: blanca.roncero@upc.edu . Tel.: +34-93-7398210. Fax:  
17 +34937398101

18

19 **ABSTRACT**

20 Bacterial cellulose was combined with wood cellulose papers in order to obtain  
21 biomaterials with increased barrier properties. For this purpose, different parameters  
22 were assessed: two producing bacterial strains (*Komagataeibacter xylinus* and  
23 *Gluconacetobacter sucrofermentans*), two paper supports to hold bacterial cellulose  
24 (filter paper and eucalyptus paper), two kinds of combined biomaterials (composite and  
25 bilayer) and two drying temperatures (90°C and room temperature). Papers with  
26 increased barrier properties (100° of water contact angle, 1220s of water drop test and  
27 air permeability  $<1\mu\text{m (Pa}\cdot\text{s)}^{-1}$ ) were obtained by the addition of bacterial cellulose to  
28 each paper support. However, due to the lower initial barrier properties of filter paper,  
29 higher improvements were produced with this paper. In addition, bacterial cellulose  
30 provided smoother surfaces with higher gloss without a detrimental effect on physical  
31 properties. Higher resistance to water absorption was obtained with *K. xylinus* possibly  
32 explained by its longer size fibers than *G. sucrofermentans*, as analysed by SEM.  
33 Smoothness and gloss were specially increased in the bilayer biomaterial although  
34 resistance to air and water were further improved in the composite. In this biomaterial  
35 drying at high temperature had a detrimental effect. SEM analysis of the products  
36 obtained showed the intimate contact among fibers of bacterial cellulose and wood  
37 paper. Results obtained show the contribution of bacterial cellulose to improve the  
38 properties of paper and its potential for the design of new added value paper products  
39 from biomass.

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46 *Keywords: bacterial cellulose, barrier properties, hydrophobicity, air permeability,*  
47 *water resistance, cellulose paper*

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## 49 INTRODUCTION

50 Cellulose is the most abundant polymer of the Earth as a main component of  
51 plant biomass. Due to its availability, it has been traditionally used as a raw material for  
52 the production of a great diversity of industrial products including, paper, cardboard,  
53 textiles, food additives and pharmaceutical products, among others. The renewed  
54 interest for biomass valorization has fostered the research for the transformation and  
55 modification of plant residues into increased value products as biofuels and  
56 biomaterials, such as nanocellulose (Tuck et al. 2012; Beltramino et al. 2015, 2016).  
57 One of the main problems found is the intimate association of cellulose with lignin and  
58 hemicelluloses in plant biomass, in lignocellulose (Gilbert 2010). Deconstruction of  
59 plant cell wall requires the development of technology to improve the separation and  
60 upgrading of its lignocellulosic components in valuable new products (Gilbert 2010;  
61 Quintana et al. 2013, 2015). Besides plants, some microorganisms can also produce  
62 cellulose. Bacterial cellulose shows identical molecular composition to plant cellulose,  
63 but it shows a major advantage: it is not associated to lignin and hemicellulose, it is a  
64 high purity polymer.

65 Comparison of plant and bacterial cellulose show several properties that are  
66 favorably increased in bacterial cellulose, among which degree of polymerization and  
67 crystallinity, that are remarkably high (Klemm et al. 2005). An important property of  
68 bacterial cellulose is biocompatibility, that together with its elevated mechanical  
69 strength has prompted its use in medical applications such as scaffold for tissue and skin  
70 regeneration, artificial blood vessels, and as thickening food additive (Lin et al. 2013).  
71 These applications are correlated to its high water holding capacity determined by a  
72 structure of well separated cellulose nanofibrils, what makes bacterial cellulose a highly  
73 porous material that can show up to more than 90% water content. However, this water  
74 holding property is notably diminished after air drying, probably as a consequence of  
75 the hydrogen-bond formation among cellulose fibrils (Klemm et al. 2005; Hagiwara et  
76 al. 2010).

77 Mechanical properties of bacterial cellulose makes it an excellent candidate for  
78 the restoration of damaged paper documents where its surface lining does not affect  
79 document readability (Santos et al. 2015, 2016a, 2017). Application of bacterial

80 cellulose as a reinforcing agent of pulps in papermaking has also been studied showing  
81 variable results depending on the pulp source (Yamanaka et al. 1989; Pommet et al.  
82 2008; Gao et al. 2010; Tang et al. 2013; Xiang et al. 2017b), while its application for the  
83 production of nanocomposites can give a diversity of high added value products such as  
84 electronic and magnetic papers (Chawla et al. 2009; Shah et al. 2013; Lim et al. 2016).

85 On the other hand, barrier properties in papers (impermeability to air, water,  
86 water vapor, oxygen, fats, microorganisms, etc.), that are especially important in the  
87 food packaging sector, are currently provided by plastic films produced from  
88 petrochemical products. However, due to the increase in social awareness regarding the  
89 harmful environmental impact and the unsustainable life cycle of these materials,  
90 research is focusing on the creation of new biomaterials from renewable resources,  
91 which besides having these advanced barrier properties, may even become  
92 biodegradable (Cusola et al. 2014). Bacterial cellulose, because of its specific  
93 properties, can fulfil these requirements (Klemm et al. 2011; Osong et al. 2016). In fact,  
94 previous works have demonstrated bacterial cellulose can decrease wettability and  
95 permeability of paper (Gao et al. 2010; Santos et al. 2017; Xiang et al. 2017b).

96 The main purpose of this work was to combine bacterial cellulose with wood  
97 cellulose in order to increase barrier properties of paper without a detrimental effect on  
98 mechanical properties. Different aspects were taken into account, such as the microbial  
99 strain, the paper type and the way of joining bacterial cellulose and paper. For this  
100 purpose, bacterial cellulose produced by two different microbial strains was firstly  
101 characterized. Then, the bacterial cellulose was combined with two wood paper types to  
102 obtain two kind of biomaterials: composites or bilayers. In the composites, bacterial  
103 cellulose was directly synthesized by the growth of the producing bacteria on the  
104 surface of filter or eucalyptus paper sheets. In the bilayers, bacterial cellulose films were  
105 previously synthesized and then coated over the surface of the paper sheets. The  
106 properties of the resulting paper products were analyzed in terms of their mechanical  
107 strength, optical and barrier properties, and SEM morphology.

## 108 **MATERIALS AND METHODS**

### 109 **Bacterial strains**

110 Strains *Komagataeibacter xylinus* (DMS-2004) and *Gluconacetobacter*  
111 *sucrofermentans* (CECT 7291) were obtained from the DSMZ German Collection of  
112 Microorganisms and Cell Cultures and from the Spanish Type Culture Collection,  
113 respectively. They were grown in Hestrin–Schramm (HS) solid medium (Hestrin 1954)  
114 in agar plates for maintenance. Suspensions of bacterial cells were obtained by gentle  
115 shaking and inoculated in flasks containing 100 mL of HS liquid medium which were  
116 incubated under static conditions for 4-7 days. Following, the surface bacterial films  
117 produced were cut in small pieces (1x1 cm) in sterile conditions and shaken in HS  
118 liquid medium at 700 rpm for 30 min to detach cells from the cellulose films. The  
119 suspensions obtained were filtered through a gauze to remove film portions, centrifuged  
120 at 4000 rpm for 10 min and, after discarding supernatants, pellets were resuspended in  
121 Ringer's solution  $\frac{1}{4}$  (NaCl 2.5 g L<sup>-1</sup>; KCl, 0.105 g L<sup>-1</sup>; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.120 g L<sup>-1</sup>; and  
122 NaHCO<sub>3</sub>, 0.05 g L<sup>-1</sup>). Optical density of the bacterial suspensions was adjusted to  
123 OD<sub>600</sub> of 0.59–0.64 with Ringer's solution  $\frac{1}{4}$  and used as inoculum for the following  
124 experiments.

### 125 **Production of bacterial cellulose films**

126 Bacterial cellulose films were produced cultivating the bacterial strains in liquid  
127 media in 150 mm diameter Petri dishes. 100 mL of HS liquid media were inoculated  
128 with 250  $\mu$ L of the bacterial suspension and incubated at 30°C for 10 days in static  
129 conditions. After growth, the produced films were soaked in 1% NaOH, incubated at 60  
130 °C for 2 h and washed with distilled water up to neutral pH. Bacterial cellulose films  
131 were dried at room temperature over Whatman filters.

### 132 **Composites and bilayers biomaterials**

133 BC was introduced in paper sheets by two different methods in order to obtain a  
134 composite or a bilayer. Two paper sheets were used in each case: commercial filter  
135 paper of 73 g m<sup>-2</sup> (Filtros Anolia 1305) or laboratory made paper sheets of 75 g m<sup>-2</sup> from

136 *Eucalyptus globulus* TCF (totally chlorine free) bleached pulp, PFI refined at 45°SR.  
137 Eucalyptus pulp was supplied by ENCE (Pontevedra, Spain).

138 Composites of bacterial cellulose films and paper were produced growing the  
139 bacterial strains on the surface of paper sheets layered on the top of solid media in 150  
140 mm diameter Petri dishes. 500 µL of the bacterial suspension were mixed with 20 mL of  
141 Ringer's solution ¼ and inoculated in 150 mL of HS solid media covered with paper  
142 sheets and incubated at 30°C for 10 days under static conditions. After growth, the  
143 composites of paper sheets and bacterial cellulose were removed, treated with NaOH,  
144 washed and dried as before at room temperature. Alternatively, they were also dried at  
145 90°C for 5 min.

146 In the bilayers, bacterial cellulose films, once washed, were layered over paper  
147 sheets and the resulting coated sheets were dried at room temperature or at 90°C as  
148 above mentioned. In this case, only the BC films from *K. xylinus*, were used.

## 149 **Paper characterization**

### 150 *Physical-mechanical properties*

151 They were determined in accordance with the standards in brackets as follows:  
152 apparent density (ISO 534:2005), tensile strength index and elongation (ISO 1924-  
153 2:1994), burst strength index (ISO 2758:2001), wet zero-span index (ISO 15361:2000)  
154 and Bendtsen roughness (ISO 8791-2:2013).

### 155 *Optical properties*

156 Pulp brightness was determined according to ISO 2470-1. Specular Gloss was  
157 determined according to ISO 8254-1:2009.

### 158 *Barrier properties*

159 Air permeance was measured with Bendtsen equipment (ISO 5636-5:2003).  
160 Hydrophobicity was measured by the water contact angle (WCA) and water  
161 impermeability by the water drop test (WDT). WCA was measured by using a  
162 Dataphysics OCA15EC contact angle goniophotometer (Dataphysics, USA), using an  
163 image capture ratio of 25 frames s<sup>-1</sup>. Following the procedure described by Cusola et al.  
164 2014 a 4 µL water drop was delivered to the sample surface. At least 8 measurements

165 were made for each sample. Water drop test (WDT) was performed on each treated  
166 paper specimen according to Tappi standard T835 om-08. Previously the paper sheets  
167 were conditioned according to ISO 187. The WDT involved placing a drop of deionised  
168 water on the surface of paper and recording the time needed for complete absorption,  
169 which was signaled by vanishing of the drop specular gloss. Fifteen measurements per  
170 treated paper sample were made and averaged.

## 171 **Scanning electron microscopy (SEM)**

172 Surface and cross-sectional SEM pictures of the different films and biomaterials  
173 obtained were taken on a JEOL JSM-6400 microscope (Japan). Samples were placed on  
174 the SEM sample holding stub by means of conductive double side sticky carbon film  
175 and coated with Au/Pd alloy prior to analysis.

## 176 **RESULTS**

### 177 **Bacterial cellulose films vs. papers from wood cellulose**

178 Several bacterial strains were tested for bacterial cellulose (BC) production on  
179 the HS standard medium. The screening includes several newly isolated and also culture  
180 collection strains. Two of them, *Komagataeibacter xylinus* and *Gluconacetobacter*  
181 *sucrofermentans* were selected as the best producers in the culture conditions assayed.

182 The selected strains were grown for 10 days at 30 °C in liquid media on Petri  
183 dishes of 15 cm diameter under static conditions. The bacterial growth produced surface  
184 cellulose films that were recovered and treated with NaOH to eliminate microbial cells,  
185 washed and dried at room temperature. Properties of the bacterial cellulose dried films  
186 obtained were analyzed and compared to those of commercial filter paper or of paper  
187 made from TCF eucalyptus pulp (Table 1). These two types of paper showed different  
188 properties in accordance to their different composition. Eucalyptus paper was smoother,  
189 had more density, higher physical properties and lower gloss than filter paper.  
190 Moreover, it had better barrier properties to air and water. The properties of the BC  
191 films produced by the two strains were quite similar and differed widely from those of  
192 paper sheets. Although BC films had lower grammage than wood papers, their  
193 mechanical properties were similar or even higher in some cases. This fact can be



194 explained by the higher density of films made of BC, due to a better conformability of  
 195 BC fibers. In fact, Chen et al. (2017) reported similar density values of films from  
 196 nanofibrillated cellulose with high strength properties, but in that case the nanocellulose  
 197 was obtained from different plants (Chen et al. 2017).

198

199 **Table 1.** Physical, optical and barrier properties of bacterial cellulose films and papers from wood fibers,  
 200 filter paper (Fp) and eucalyptus paper (Eu)

	Bacterial cellulose films		Papers from wood fibers	
	<i>K. Xylinus</i>	<i>G. sucroferm.</i>	Fp	Eu
Grammage (g m <sup>-2</sup> )	10.7±2.1	8.1±0.7	71.4±1.4	76.2±0.8
Thickness (µm)	9.7±1.3	9.3±1.3	154±4.9	115±1.0
Apparent density (g cm <sup>-3</sup> )	1.1±0.1	0.9±0.1	0.5±0.0	0.7±0.0
Tensile strength index (N·m g <sup>-1</sup> )	18.1 ± 5.2	61.7 ± 1.5	34.0 ± 3.2	45.0 ± 7.2
Burst strength index (kN g <sup>-1</sup> )	6.4 ± 0.4	1.2 ± 0.9	1.8 ± 0.2	3.0 ± 0.1
Elongation (%)	0.8 ± 0.4	ND	2.0 ± 0.5	2.7 ± 0.2
Wet Zero-Span index (N·m g <sup>-1</sup> )	126±26	114 ± 4	110 ± 1	106 ± 3
Gloss (%)*	31.0 ± 6.0	32.5 ± 3.3	17.0 ± 0.3	0.2±0.2
Brightness (%)*	81.4 ± 0.8	82.5 ± 0.7	86.3 ± 0.1	85.0 ± 0.6
Bendtsen roughness (mL min <sup>-1</sup> )*	24 ± 9	30 ± 7	1823 ± 211	993± 54
Bendtsen Air Permeance (µm (Pa·s) <sup>-1</sup> )*	1.3±0.1	1.1±0.5	52.9±1.5	7.4±1.3
WDT (s)*	4121±300	4823±247	1.7±0.3	10.7±1.7
WCA (°)*	48.8±10.9	38.6±0.8	24.0±2.3	33.8±7.0

201 \* Properties measured in the upper face

202

203 The more compact structure of BC provided dense films with a smoother surface  
 204 (lower Bendtsen roughness), and therefore higher gloss (Table 1). However, the most  
 205 remarkable difference with paper sheets was the strongly increased barrier properties to  
 206 air and water. Although air permeance was lower in eucalyptus paper than in filter  
 207 paper, it was significantly much lower in BC films. They also showed a remarkable  
 208 increased water drop test that raised from 10 s in eucalyptus paper to more than 4000 s  
 209 in BC films. The water contact angle showed also higher values in BC films.  
 210 Comparing with other reports in which nanocellulose from plant cellulose was used, it  
 211 was found that nanocellulose provide lower air permeability (Syverud and Stenius 2009;

212 Gicquel et al. 2017; Herrera et al. 2017) and similar values of WCA (Beltramino et al.  
213 2015).

214 The results showed the high barrier properties of the films made of bacterial  
215 cellulose. The high resistance to water absorption and to air penetration of the BC dried  
216 films is an important trait that can be applied to enhance barrier properties of paper  
217 sheets, especially in food packaging, in order to replace petrol-based packaging by  
218 biodegradable products. For this reason, in order to provide these properties to final  
219 papers products, two kinds of biomaterials were made combining BC and paper sheets:  
220 composites and bilayer.

## 221 **Bacterial cellulose-paper composites**

### 222 *Mechanical and optical properties of composites*

223 To evaluate the contribution of BC to the properties of paper made from wood  
224 pulp, composites of the two types of cellulose were made. For this purpose, the BC  
225 producing strains were grown on the surface of paper sheets (filter or eucalyptus)  
226 soaked on the top of solid media in 150 mm petri dishes. The paper sheets covered with  
227 the bacterial growth were recovered and treated with alkali in the same conditions as  
228 above. They were dried at room temperature or, alternatively, at 90 °C and their physical  
229 properties analyzed (Table 2).

230 **Table 2.** Physical and optical properties of Composites made of filter (Fp) or eucalyptus (Eu) papers and  
231 bacterial celluloses dried at room temperature

	Composite Fp		Composite Eu	
	<i>K. xylinus</i>	<i>G. sucroferm</i>	<i>K. xylinus</i>	<i>G. sucroferm</i>
Tensile strength index (N·m g <sup>-1</sup> )	37.7 ± 1.5	39.5 ± 0.5	44.7 ± 2.7	46.5 ± 1.7
Burst strength index (kN g <sup>-1</sup> )	1.8 ± 0.1	2.3 ± 0.4	2.4 ± 0.140	2.7 ± 0.4
Elongation (%)	1.8 ± 0.1	2.7 ± 0.2	2.2 ± 0.2	3.5 ± 0.254
Wet Zero-Span index (N·m g <sup>-1</sup> )	108 ± 4	101 ± 22	90 ± 1	75 ± 6
Gloss (%)*	31.9 ± 1.4	31.5 ± 3.8	23.3 ± 2.2	22.9 ± 1.6
Brightness (%)*	74.3 ± 0.5	79.0 ± 0.3	71.1 ± 0.2	79.2 ± 0.3
Bendtsen roughness (mL min <sup>-1</sup> )*	1372 ± 171	1374 ± 223	945 ± 50	826 ± 128

232 \* Properties measured in the upper face

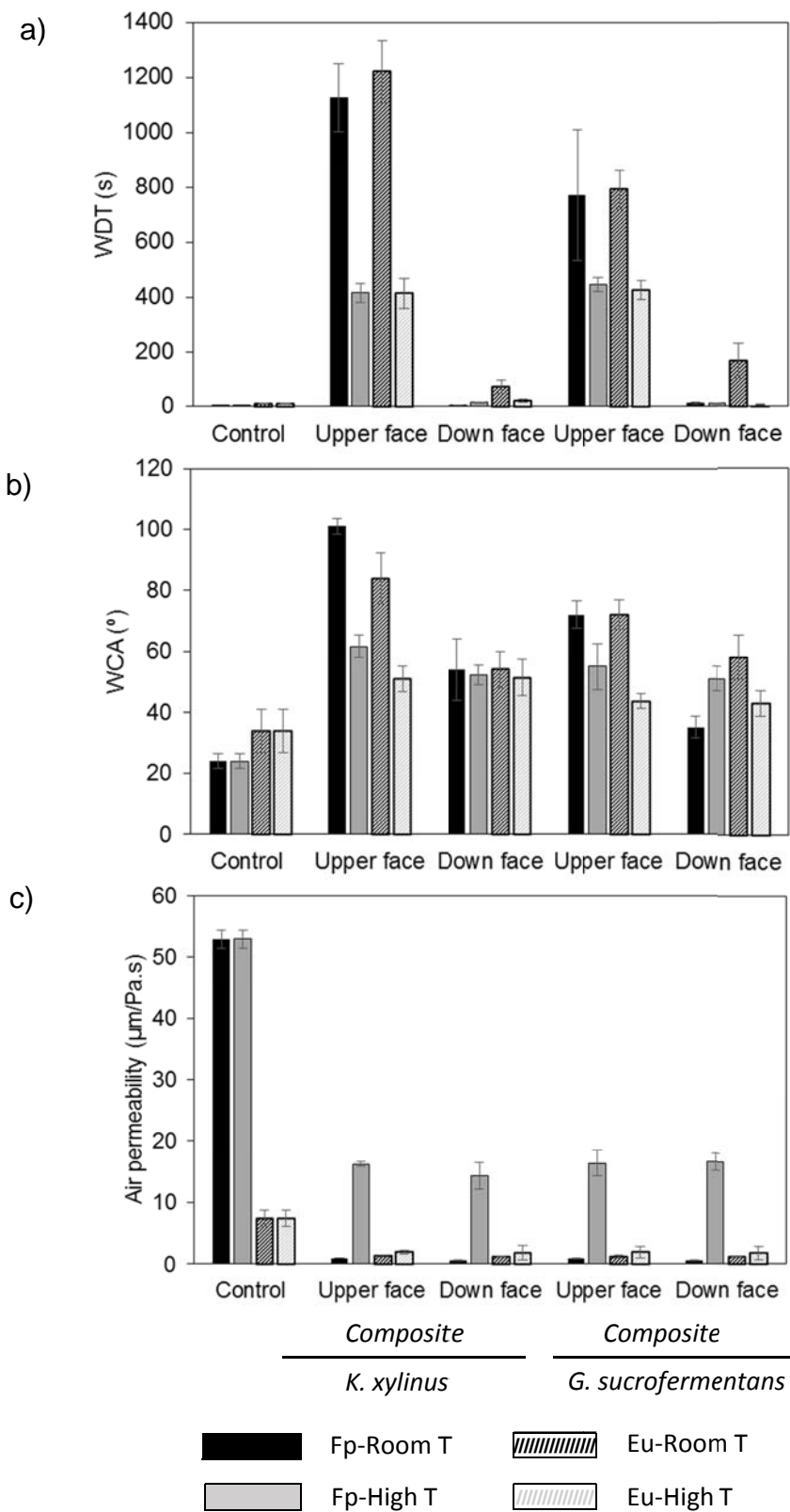
233

234 Physical properties (grammage, thickness and apparent density) of papers were  
235 not significantly affected by the addition of BC. In general, mechanical properties of the  
236 BC-paper composites showed similar values or a small increase than control paper  
237 sheets (Table 1). This increase was slightly higher with *G. sucrofermentans* than with *K.*  
238 *xylinus*.

239 Optical properties were determined on the upper face of the composites, that  
240 covered by the BC (Table 2). Gloss is an important property in the printing paper  
241 industry. Composites reached a notably higher gloss than their control samples, showing  
242 similar increased values with the two types of BC. Santos et al. (2017) reported that  
243 nonglossy papers can show a noticeable increment in their specular gloss when  
244 reinforced with BC, in accordance with our results. Interestingly, a higher increase in  
245 gloss than in papers coated by cellulose nanocrystals from biomass (Gicquel et al. 2017)  
246 was obtained here with bacterial cellulose. Composites of filter paper showed also  
247 remarkable increase of whiteness (data not shown). Brightness determination revealed a  
248 decrease in this property in all composites. Composites with *K. xylinus* had the lowest  
249 brightness values.

#### 250 *Barrier properties of composites*

251 The BC-paper composite sheets showed a notably lower wettability than the  
252 control paper sheets (Fig. 1), although not as low as the bacterial cellulose films (Table  
253 1). WDT was determined on the two sides of the composites, the upper face, covered by  
254 BC, and the down face of the sheets. The WDT values of the upper face of the  
255 composites were remarkably higher in all samples (Fig. 1a). It was increased from 2-10  
256 s in control paper sheets not covered by BC to values ranging from 414 to 1220 s in the  
257 BC-pulp composites. Down face of the composite sheets showed much lower increase  
258 in water drop test values, indicating higher wettability of this face of the composites,  
259 probably because the lack of bacterial cellulose penetration among pulp fibers in this  
260 side. Regarding the influence of the drying temperature on the properties of the  
261 composites, a detrimental effect of temperature was found, as water drop value of  
262 samples dried at room temperature was approximately two times that of parallel samples  
263 dried at 90 °C.



265

267 **Fig. 1.** Barrier properties to water of Composites made of filter (Fp) or eucalyptus papers (Eu) and  
 268 bacterial celluloses dried at room or high temperature. a) WDT; b) WCA; c) Air Permeability

267

268 To evaluate the hydrophobicity of the composites, the water contact angle was  
269 also analyzed. The results showed an increased water contact angle of all composites,  
270 that exhibited up to 3 fold increase compared with control paper sheets (Fig 1b).  
271 Moreover, the differences between upper and down faces of the composites were  
272 minimized. The drying temperature also influenced in water contact angle, with higher  
273 results for the samples dried at room temperature.

274 The results found revealed that the composites of bacterial cellulose and paper  
275 sheets have a diminished capacity of water absorption, indicating an increased barrier  
276 property to water. To evaluate the barrier property to a different matter, air, the air  
277 permeability was analyzed. Composites containing filter paper showed a high decrease  
278 in permeability that diminished from the values corresponding to a high permeable  
279 control filter (52.9  $\mu\text{m}/\text{Pa}\cdot\text{s}$ ) to a very closed paper (0.53-0.94  $\mu\text{m}/\text{Pa}\cdot\text{s}$ ) when the  
280 composites were dried at room temperature. A lower effect was produced with  
281 eucalyptus sheets (from 7.4 to 1.11  $\mu\text{m}/\text{Pa}\cdot\text{s}$ ). Drying at high temperature gave also less  
282 permeable composites although permeability was decreased in lower extent, especially  
283 with filter paper. The lower air permeability was probably due to the small BC  
284 fragments filling the gaps between wood fibers and increasing the affinity between  
285 them. Controlling the permeability of substances through the packaging is also very  
286 important in food packaging in order to increase the shelf life of the product. In fact,  
287 Tabarsa et al., 2017 also found a decrease in porosity combining BC and softwood  
288 fibers, but mixing the fibers with BC before sheet formation.

### 289 *K. xylinus* vs. *G. sucrofermentans*

290 *K. xylinus* has been applied in previous works to increase the Young's modulus  
291 of composites made with cellulose acetate butyrate (Gindl and Keckes 2004) or with  
292 phenolic resins (Nakagaito et al. 2005), or to modify the surface of natural fibers to  
293 improve composite properties (Pommet et al. 2008). It has been also used to increase the  
294 physical properties of papers resulting from mixing the BC with wood fibers (Gao et al.  
295 2011; Tabarsa et al. 2017; Xiang et al. 2017a), but in a different manner as in the  
296 present paper and with different results. However, fewer works have been reported with  
297 *G. sucrofermentans*. A similar composite was performed by Santos et al. (2015, 2016a,

298 b, 2017) in order to reinforce degraded papers. In this case, no variation in physical  
299 properties and a reduction of wettability was also found.

300 The results obtained in this research revealed that the two bacterial strains  
301 provided different properties in some cases. In contrast with the similar barrier  
302 properties of the BC films of the two producing bacteria, composites containing *K.*  
303 *xylinus* cellulose gave higher values of water barrier properties (Fig.1) than composites  
304 with *G. sucrofermentans* cellulose (increase of up to 1100 s of WDT and 77° the WCA  
305 with the former vs. increase of 760 s WDT and 48° WCA with the latter in the case of  
306 filter paper, and a similar behavior in eucalyptus paper). On the other hand, in both  
307 paper supports, the two BC partners of the composites made a similar contribution to air  
308 permeability, as similar values were found for *K. xylinus* and *G. sucrofermentans*  
309 composites.

310

### 311 **Bacterial cellulose-paper bilayer**

#### 312 *Mechanical and optical properties of bilayer biomaterial*

313 The BC-pulp composites analyzed were produced by the direct growth of the  
314 cellulose producing bacteria on the surface of paper sheets. The rationale of this  
315 methodology was that the fibers of bacterial cellulose would probably grow intermixed  
316 among pulp fibers, making a compacted composite, which as we have shown, would  
317 exhibit an increased resistance to fluid penetration. The good results obtained made us  
318 to evaluate a different strategy to combine pulp and bacterial cellulose in a sheet. For  
319 this purpose, previously produced BC films were layered on paper sheets and the bilayer  
320 sheets were dried by the same procedure as above mentioned. We only used BC films  
321 from *K. xylinus*, which gave best results as previously shown.

322 Similar to that previously obtained in composites, physical and mechanical  
323 properties were not significantly affected in the bilayer material (Table 3). On the other  
324 hand, gloss was strongly increased, even more than in the composite. Brightness was  
325 decreased but in a lower extent than in the composite and roughness was strongly  
326 decreased, especially in filter paper. A high smoothness is a required property in  
327 printing applications and essential in printed electronics. It has been reported that other  
328 kind of nanocelluloses from biomass applied on paper surface as coating treatment also

329 provide some smoothness increase (Brodin et al. 2014; Gicquel et al. 2017; Herrera et  
330 al. 2017).

331 **Table 3.** Physical and optical properties of Bilayers made of filter (Fp) or eucalyptus (Eu) papers and  
332 bacterial cellulose from *K. xylinus* dried at room temperature

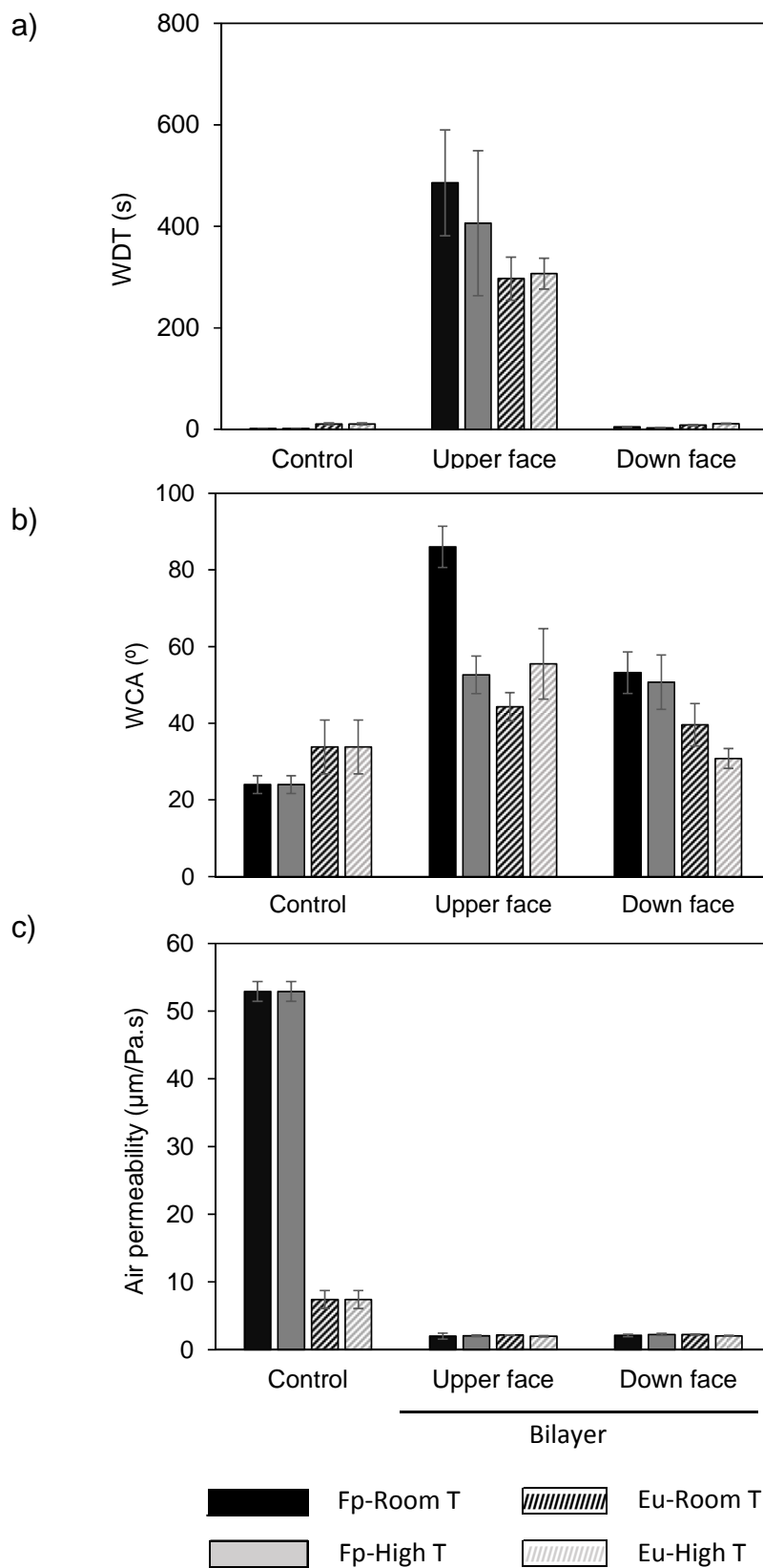
	Bilayer Fp	Bilayer Eu
Tensile strength index (N·m g <sup>-1</sup> )	30.8 ± 1.7	39.4 ± 1.6
Burst strength index (kN g <sup>-1</sup> )	2.0 ± 0.0	2.3 ± 0.2
Elongation (%)	1.9 ± 1.6	1.9 ± 0.2
Wet Zero-Span index (N·m g <sup>-1</sup> )	95 ± 4	97 ± 2
Gloss (%)*	49.2 ± 2	46.4 ± 0.4
Brightness (%)*	80.0 ± 1.0	81.6±0.8
Bendtsen roughness (mL min <sup>-1</sup> )*	680 ± 158	517 ± 41

333 \* Properties measured in the upper face

### 334 *Barrier properties of bilayer biomaterial*

335 The barrier properties of the bilayer sheets were determined on both sides (Fig.  
336 2). Wettability of bilayer sheets was much lower than control paper sheets. Water drop  
337 test was again notably increased to values around 490 to 300 s in filter and eucalyptus  
338 papers, respectively. The values obtained were much lower than those of the  
339 corresponding BC-paper composites. No significant differences were obtained at room  
340 or high temperature. Similar to that observed in the composites, no effect was produced  
341 in the down face of the bilayer. In agreement with the water drop values, the water  
342 contact angle of bilayer sheets was also increased in the upper face to 86° and 44° in  
343 filter and eucalyptus papers, respectively. By contrast with that obtained with the WDT,  
344 the water contact angle was increased in the down face of the bilayer made with filter  
345 paper, although in a lower extent than in the upper face (53°). The temperature used for  
346 drying, room or hot, did not make an important difference in wettability of bilayers, it  
347 was only reduced in the upper face of filter paper.

348



349

350 **Fig. 2.** Barrier properties to water of Bilayer made of filter (Fp) or eucalyptus papers (Eu) and bacterial  
 351 cellulose from *K. xylinus* dried at room or high temperature. a) WDT; b) WCA; c) Air Permeability



352

353           The barrier property to air, measured as air permeability, was strongly decreased  
354 in the bilayer biomaterials, especially in the case of filter paper. Similarly to that found  
355 in the composites no differences between the upper and lower face were observed, and  
356 no effect of the drying temperature was produced.

357           Therefore, interesting results were found concerning the barrier properties to air  
358 and water with paper and BC. Nanocelluloses from plants instead of bacteria have also  
359 been used to improve these properties. Several authors (Syverud and Stenius 2009;  
360 Aulin et al. 2010; Lavoine et al. 2014b) obtained a complete reduction of air  
361 permeability when nanofibrillated cellulose was applied as a surface layer on paper  
362 sheets. However Lavoine et al. 2014a found that nanofibrillated cellulose did not  
363 increase the barrier property to water. Lower knowledge exists about the barrier  
364 properties that cellulose nanocrystals coated on papers may provide. Recently, Gicquel  
365 et al 2017 reported that papers coated with cellulose nanocrystals can strongly reduce its  
366 air permeability maintaining the mechanical properties. One of the problems associated  
367 with coating with this kind of nanocellulose is that the surface obtained is brittle and the  
368 coat is split along the substrate fiber (Gicquel et al. 2017).

### 369 *Composite vs. bilayer biomaterial*

370           Two kinds of biomaterials (composite and bilayer) have been constructed  
371 combining wood fibers and bacterial cellulose produced by *K. xylinus*. Physical  
372 properties of papers were not adversely affected by the addition of bacterial cellulose in  
373 any case. Previous works (Gao et al. 2010; Tabarsa et al. 2017; Xiang et al. 2017a)  
374 reported an increase in physical properties in softwood or sugarcane bagasse fibers with  
375 *K. xylinus*. Some of these authors also stated that the amount of bacterial cellulose  
376 incorporated could affect the increases in physical properties. For example, Xiang et al.  
377 (2017a) specified that BC has to be introduced at low doses (lower than 1%) whereas  
378 Tabarsa et al.(2017) and Gao et al. (2010) found that physical properties of the sheets  
379 increased with the bacterial cellulose dosage. According to the grammage increase (data  
380 not shown), we determined that the amount of bacterial cellulose incorporated in our  
381 biomaterials was around 15%, which is similar to that used in these papers. An  
382 explanation of the different behavior found may be explained by the way in which BC

383 was introduced in vegetal fibers: in the previous works quoted, BC was disintegrated  
384 and mixed with fibers before sheet formation. Moreover, the wood fibers used in our  
385 work were refined, what probably made more difficult to increase the physical  
386 properties. In fact, Surma-Slusarska et al. (2008) also obtained a reduction in some  
387 physical properties when they combined BC and pine or birch fibers, obtaining a  
388 bilayer. Mechanical properties can also be increased by the addition of nanofibrillated  
389 cellulose as an additive in papermaking (Boufi et al. 2017).

390 Concerning brightness property, lower values were obtained in composites than  
391 in bilayer. This could be related with the highest roughness of composites structures. In  
392 fact, Gicquel et al. (2017) found that when the roughness increased, brightness  
393 decreased in their study in which paper samples were coated with nanocellulose.  
394 Moreover, Brodin et al. (2014) stated that the addition of nanofibrillated cellulose in the  
395 paper reduced the light scattering coefficient and the brightness of the sheets.

396 BC provided smoother surfaces with higher gloss in the upper face of both  
397 biomaterials. These properties were more improved in the bilayer biomaterial.  
398 Smoothness is an important factor that determines the good paper printability. However,  
399 barrier properties to water and air were much higher increased in composites. In the  
400 composite made with filter paper WDT and WCA increased up to 1120 s and 77° with  
401 BC whereas these increases were 480 s and 62° in the bilayer. Similarly, permeability  
402 was decreased 98% in the composite vs. 96% in the bilayer.

403 The temperature used for drying the biomaterials (room or 90°C) had some  
404 influence on the final properties, that was different in the composites or bilayer  
405 materials. Whereas in the composites a detrimental effect in barrier properties was  
406 produced by drying at high temperature, no significant effect of temperature was  
407 produced in the bilayer materials. In both cases, the wettability was strongly reduced in  
408 the lower faces, because the lack of bacterial cellulose penetration among pulp fibers in  
409 this side, whereas the air permeability was not affected. The heterogeneous network  
410 structure of composite, formed by vegetal fibers (macro-material) and bacterial  
411 cellulose (nano-material) could be the reason of the different effect of temperature  
412 drying in final properties. Before the drying treatment, the composite has two wet  
413 materials with different size, and with different drying kinetics. According to the drying  
414 theory of porous materials, when the drying temperature is higher, the evaporation rate

415 increases, that is, the drying kinetics is faster. Then the differences between the size and  
416 the temperature during the drying treatment of the composite structure gives rise to a  
417 different behavior of cellulosic fibers and bacterial cellulose, and therefore to a different  
418 final dried structure.

419 The results show that the adhesion of bacterial cellulose films to the surface of  
420 paper sheets in the bilayer gives rise to novel sheets with decreased wettability, and  
421 decreased air permeability. However, the increase in barrier properties is much lower  
422 than that obtained when bacterial cellulose and paper fibers are more intensely  
423 interconnected in a composite.

#### 424 *Eucalyptus vs. filter paper*

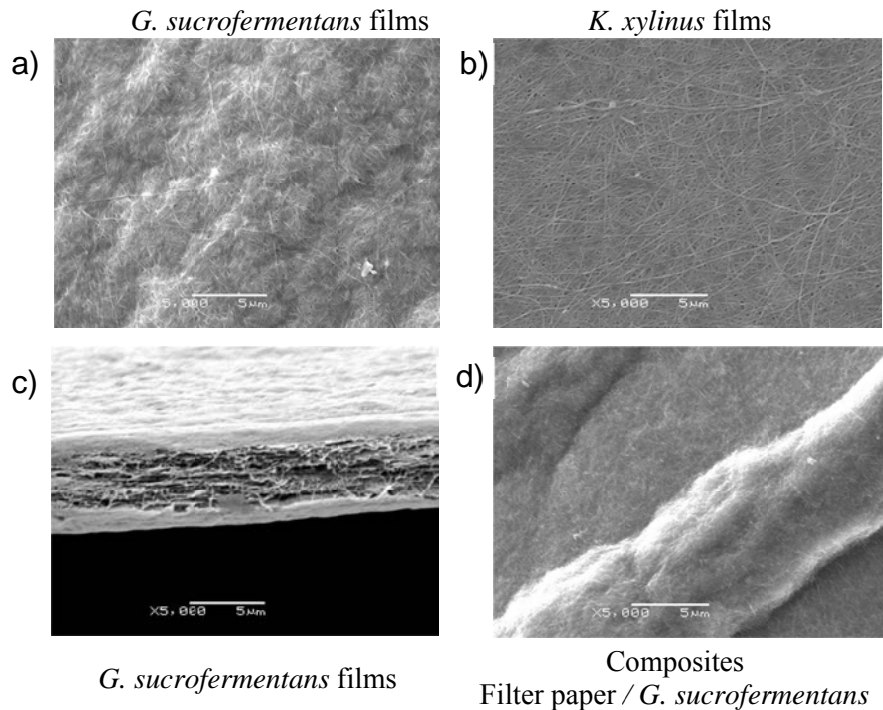
425 Values of barrier properties obtained in composites with each kind of paper  
426 support were very similar. However, the increases produced in each case were different.  
427 The increase in WDT was similar in both paper types (1120 s vs. 1210 s), whereas the  
428 WCA increase was slightly higher in filter paper (77° vs. 50° in eucalyptus). Finally, the  
429 air permeability decrease was also greater in filter paper (98%) than in eucalyptus  
430 (83%). These results suggest that the composition of the paper sheets used as support to  
431 hold the bacterial growth gave also some influence.

432 In the bilayer biomaterial, a similar effect than in composites was produced. The  
433 WDT and WCA increases were higher in filter paper (480 s and 62°) than in eucalyptus  
434 (290 s and 10°). Determination of air permeability showed that bilayers made of  
435 bacterial cellulose and filter paper exhibited a notable decrease of permeability (96%),  
436 while when the paper component of the bilayer was eucalyptus a lower effect was  
437 produced (71%). In fact, this effect may be explained by the lower initial barrier  
438 properties of filter paper, what made it easier to improve them.

#### 439 **Scanning Electron Microscope analysis**

440 Microscopic observation showed a different surface aspect of *K. xylinus* and *G.*  
441 *sucrofermentans* BC films, although both of them are formed by a dense net of thin  
442 cellulose fibers. Those of *K. xylinus* showed longer size fibers while *G. sucrofermentans*  
443 films showed frequent short fibers. These differences may explain the different behavior  
444 of bacterial cellulose in the composites, since barrier properties to water were more

448 increased with *K. xylinus*. Cross section of the BC films show that BC fibers are more  
 449 abundant and intensely connected at the surfaces, while they are more dispersed inside  
 450 the films (Fig. 3a-c).

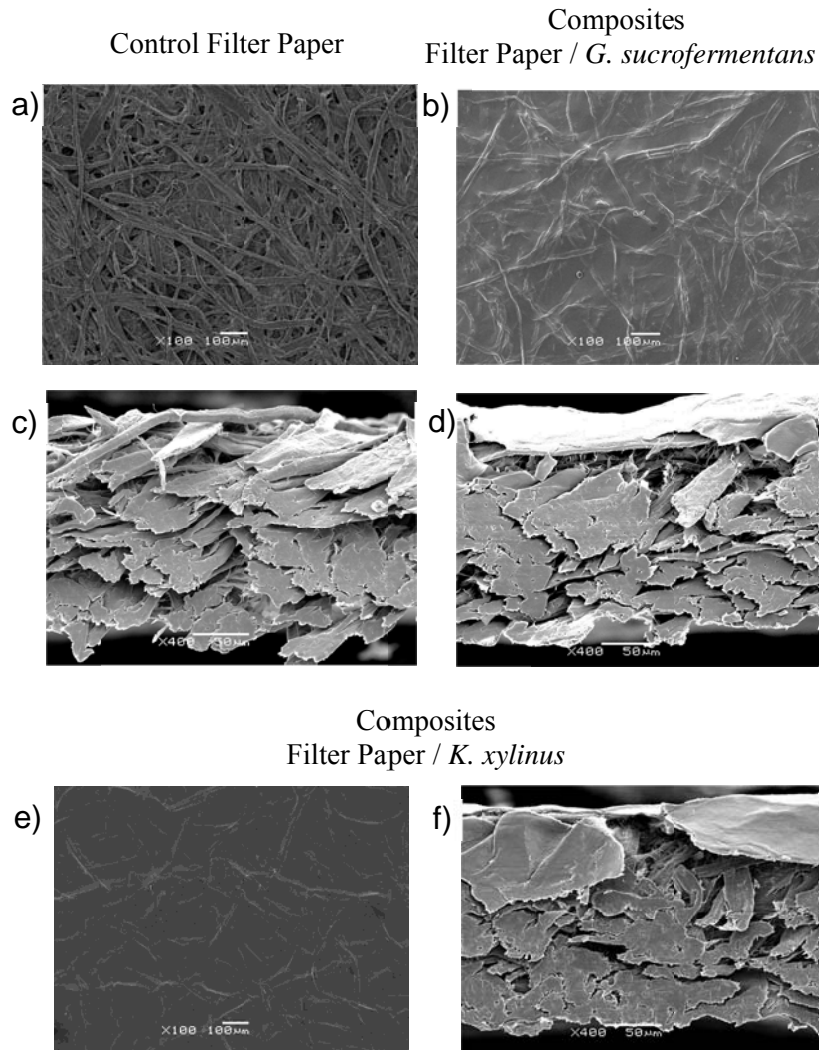


449

451 **Fig. 3** SEM images of bacterial cellulose films from *G. sucrofermentans* (a, c) and *K. xylinus* (b).  
 452 Composite of filter paper and *G. sucrofermentans* (d)

452

464 SEM analysis of BC-paper composites showed how the two types of cellulose  
 465 fibers (bacterial and pulp) are interconnected. Paper fibers, of much thicker width, are  
 466 covered by thin BC fibers making a compact material (Fig 3d). Analysis of composite  
 467 surface shows it is covered by BC fibers that fill the space among pulp fibers making an  
 468 apparently smooth and closed surface, in accordance with the increase in barrier  
 469 properties found (Fig 4a, b). Cross section of the composites visualizes also the thin  
 470 layer of BC fibers from *G. sucrofermentans* growing mainly on the surface of the  
 471 composite (Fig 4c, d). A similar effect was found with the composite obtained from *K.*  
 472 *xylinus* (Fig 4e, f). The low thickness of the BC layer on paper sheets justifies that  
 473 thickness of composites or bilayers was not greatly modified. The images of composites  
 474 show a compact structure, which made the surface of biomaterials more hydrophobic  
 475 than the original paper surface.



466

468 **Fig. 4** SEM images of control filter paper (a, c); composite of filter paper and *G. sucrofermentans* (b, d);  
 469 composite of filter paper and *K. xylinus* (e, f)

## 469 Conclusions

476 Hydrophobic and non-porous papers can be obtained combining wood cellulose  
 477 papers with a natural, biodegradable material: bacterial cellulose. Results show that this  
 478 effect depends on the bacterial strain used, on the kind of paper used and in the way BC  
 479 is incorporated into paper supports. Thus, BC from *K. xylinus* that presented longer size  
 480 fibers had stronger effect in reducing wettability of composites than *G. sucrofermentans*  
 481 that showed frequent short fibers. Barrier properties were more increased in filter paper,  
 482 probably due to its worse initial properties. Finally, although smoother surfaces with

476 higher gloss can be obtained in the bilayer in comparison with the composite, BC-paper  
477 fibers are more intensely connected in a composite, providing higher barrier properties  
478 to air and water than in a bilayer biomaterial.  
479  
480

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