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Pineapple Agroindustrial Residues for the Production of High Value Bacterial Cellulose with Different Morphologies

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ABSTRACT: Bacterial cellulose (BC) with different morphologies was biosynthesized by *Gluconacetobacter medellinensis* strain under static and dynamic culture conditions using sugar cane juice and pineapple residues as sources of carbon and other nutrients. Hestrin and Schramm's standard culture medium was used as reference. The fermentation condition and resulting yield, physico-chemical properties, and morphology relationships of obtained cellulose were analyzed. Pineapple agroindustrial residues can be envisaged as an inexpensive and sustainable alternative resource for the production of different BC morphologies. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41237.

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INTRODUCTION

As the primary components of plant cell walls, cellulose is a major constituent of the vegetable biomass and represents the most abundant biopolymer on Earth. Cellulose can also be produced by some animals and in particular by some microorganisms, including bacteria belonging to *Gluconacetobacter, Rhizobium, Sarcina, Agrobacterium, Alcaligenes* genera, which have the ability to synthesize high value cellulose known as BC or microbial cellulose.[1–4]

The biosynthesis of cellulose achieved by the activity of microorganisms leads to a more pure cellulose than that obtained from other sources, which gives unique features to the BC such as high degree of crystallinity and high tensile strength.[5,6] However, in terms of chemical structure, BC is identical to that produced by plants. Moreover, BC possesses higher surface area compared with cellulose from plants because is extracellularly synthesized into 3D networks of well-separated nano- and microfibrils. This structure is able to hold large amount of water, about 98–99% (w/w), maintaining a high degree of structural coherence. The high purity and unique mechanical properties of BC can be exploited for multiple applications.[7,8]

The biosynthesis of BC by microorganisms is directly influenced by the composition (nutrients nature) and conditions (static and dynamic) of culture medium. Conventional sources based on carbon and other nutrients (sugars such as glucose, fructose, and sucrose) are used for microbial fermentation.[9–11] Recently, unconventional carbon sources based on alternative substrates, in particular agroindustrial wastes, with low cost and reduced environmental impact have been investigated to produce high value BC.[12–18] Sugar cane and pineapple wastes are particularly interesting due to they are rich in carbon source and other nutrients, making it attractive for fermentation.[19]

Respect to culture conditions, there are two main culture methods to synthesize extracellular BC. Static culture conditions produced a gelatinous cellulose membrane at the air/liquid interface on the surface of culture medium. On the other hand, BC resulting from agitated conditions was synthesized in various forms and shapes suspended in the culture medium.[3,4,20–24]

In this work, two different culture medium conditions (static and dynamic) have been studied to identify the differences in the biosynthesis process of BC. Moreover, agroindustrial pineapple waste and sugar cane juice as sources of carbon and other nutrients have been successfully used. The morphology, physico-chemical properties, cellulose yield, and the water holding capacity of obtained materials have been extensively evaluated

and related with fermentation conditions and also, compared with those obtained using the standard Hestrin and Schramm's medium.

MATERIALS AND METHODS

Materials and Reagents

The materials for HS standard culture media, D-Glucose ($C_6H_{12}O_6 \geq 99.0\%$), yeast extract ($\geq 89.0\%$), disodium hydrogen phosphate ($Na_2HPO_4 \geq 99.0\%$) and citric acid ($C_6H_8O_7 \geq 99.5\%$) were purchased from Sigma Aldrich and peptone ($\geq 85\%$) was supplied by Panreac. P medium was prepared with commercial sugar cane and pineapple residues that were collected from household generated waste. *Gluconacetobacter medellinensis* bacteria strain was isolated from vinegar broth fermentation[25] and kindly supplied by New Materials Research Group, Pontificia Bolivariana University of Medellín, Colombia.

BC Biosynthesis

BC in membrane and sphere shapes were produced in our laboratory using *Gluconacetobacter medellinensis* bacteria strain employing different culture media, i.e., pineapple solid waste (P)[25,26] and Hestrin-Scharmm (HS)[27] and different culture conditions, static (S) and dynamic (D).

The initial strain solution was inoculated in different nutrient media with a pH of 3.5. In the case of P culture medium, a mixture of 93.43 g sugar cane juice prepared dissolving sugar cane in water and 40 g of pineapple peel juice obtained from washed, crushed and squeezed agroindustrial pineapple peel residue, was prepared in 1 L of water. The sugar composition of P medium, determined by high permeation chromatography, is 82.6 (g/L) sucrose, 4.8 (g/L) glucose, 5.04 (g/L) fructose, < 0.49 (g/L) maltose, < 0.39 (g/L) maltotriose. For HS culture medium, 20 (g/L), glucose, 5 (g/L) peptone, 5 (g/L) yeast extract, 2.7 (g/L) Na_2HPO_4, and 1.1 (g/L) citric acid were dissolved in 1 L water. Both culture media were sterilized by autoclave at 120°C during 15 min. The carbon (C), hydrogen (H), and nitrogen (N) content of each medium was determined before inoculum addition by CHN elemental analysis and was found to be 41.20% C, 6.30% H, and 0.43% N in P medium and 35.60% C, 6.80% H, and 0.68% N in HS culture medium, for BC obtained from unconventional an conventional culture media, respectively. Under static conditions in both culture media, a BC pellicle was obtained after 13 days of incubation at 28°C using 1% (v/v) inoculum. These cellulose samples were named as BC-P/S and BC-HS/S, for BC obtained in unconventional and conventional culture media, respectively. Under dynamic conditions, 250 mL of both media were incubated using also 1% (v/v) inoculum. The fermentation process was carried out at 28°C at 100 rpm for 7 days obtaining sphere-like BC, BC-P/D, and BC-HS/D in unconventional and conventional culture media, respectively.

The sphere-like or membrane BC samples, harvested in both culture media, were rinsed with water and subsequently treated with 2% (wt/v) KOH solution for 24 h at room temperature to remove non-cellulosic compounds and then thoroughly washed with water until total neutralization. Purified samples were freeze-dried prior to further characterization.

CHARACTERIZATION

Culture Composition

The sucrose, fructose, glucose, maltose, and maltotriose sugars concentrations (g/L) in P medium were analyzed by High Performance Liquid Chromatography (HPLC), using a PerkinElmer chromatograph equipped with an isocratic pump, column oven and refractive index detector. The separation was carried out at 40°C in a Brownlee Analytical Amino 5 μm (100 x 4.6 mm) column, from PerkinElmer. 1 mL/min of Acetonitrile:H_2O (75 : 25) was used as mobile phase. Samples were filtered using cellulose acetate filters of 0.45 μm pore dimension and 20 μL of sample was injected.

Elemental analysis was carried out in a Euro EA Elemental Analyzer (CHNS) from EuroVector to independently measure C, H, and N content of culture media.

BC Yield

For yield measurements, BC samples were weighted and values in g/L were expressed respect to the volume of culture media. To compare the yield of static and dynamic conditions, a sample from static culture was taken after seven days of incubation period. Each yield was measured five times.

Polymerization Degree (PD)

The PD of the different BC samples was calculated through viscosity value $[\eta]$ using the procedure described in ASTM D1795 standard method.[28] All experiments were performed using an Ubbelholde capillary viscosimeter and using cupriethylendiamine (CED) as solvent. The viscosity values were converted to PD according to the following equation[29]:

$$[\eta] = 2.42\mathrm{PD}^{0.76} \quad (1)$$

Three samples of each system were assessed.

Water Holding Capacity (WHC)

The freeze-dried BC samples were previously vacuum dried (W_{dry}). BC samples were immersed in deionized water until a constant weight was reached, rehydrated weight (W_{wet}). The water content was calculated with the following equation:

$$\mathrm{WHC}(\%) = \left(\frac{W_{wet} - W_{dry}}{W_{dry}}\right) \times 100 \quad (2)$$

All samples were measured in triplicate.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FITR was used to identify functional groups present in the BC samples. For this purpose, a Nexus Fourier transform infrared spectrophotometer from Nicolet in the range of 4000–700 cm^{-1} was used. All spectra were recorded with the accumulation of 128 scans with a resolution of 4 cm^{-1}.

Solid-State CP/MAS ^{13}C Nuclear Magnetic Resonance (^{13}C-NMR)

^{13}C-NMR solid state spectra were performed at room temperature on a Bruker 400 WB Plus spectrometer. Spectra were collected using a 4 mm CP-MAS probe at a spinning of 10,000 Hz. CP/MAS ^{13}C-NMR spectra of solid samples were recorded for 12 h using the standard pulse sequence at 100.6 MHz, a time

Table I. Polymerization Degree, Crystallinity Index, and the Yield of the BC Samples Obtained in Static and Dynamic Culture in the P and HS Media

	Static culture		Dynamic culture	
	P	HS	*P*	HS
Yield (g/L)	3.24 ± 0.42 (3.97 ± 0.36)[a]	1.94 ± 0.10 (2.15 ± 0.52)[a]	0.82 ± 0.26	0.44± 0.01
PD	2970 ± 99.00	2780 ± 28.28	2692 ± 51.62	2658 ± 7.10
CI (%)	84	88	83	75

[a] Between brackets, the yield determined after 13 days of incubation.

domain of 2K, a spectral width of 29 kHz, a contact time of 1.5 ms and an inter-pulse delay of 5 s.

X-ray Diffraction (XRD)

XRD diffraction patterns were measured using PHILIPS X'Pert Pro diffractometer, in theta-theta configuration secondary monochromator with CuKα ($\lambda = 0.154$ nm) and a solid state pixel detector, operating at 40 kV with a filament of 40 mA. The diffraction data were collected from 2θ values 5° to 40°, where θ is the angle of incidence of the X-ray been on the sample.

The degree of crystallinity (crystallinity index, CI) was calculated from the diffracted intensity values using the method described by Segal et al.[30]:

$$\mathrm{CI}(\%) = \frac{I_{200} - I_{\mathrm{am}}}{I_{200}} \quad (3)$$

where I_{200} is the maximum intensity of the (200) lattice diffraction at $\sim 2\theta = 22.7°$ and I_{am} is the intensity scattered by the amorphous part of the sample (the location of the amorphous material intensity considered in this work was at $\sim 2\theta = 18°$).

***Scanning* Electron Microscopy (SEM)**

SEM was used to observe the morphology of biosynthesized BC in different conditions. The samples were coated with gold/palladium using an ion sputter coater and observed with a Jeol JSM 6400 scanning microscope operated at 20 kV.

RESULTS AND DISCUSSION

The yields of BC produced in the P and HS culture media under static and dynamic culture conditions are summarized in Table I. As it can be observed, the yields of P culture medium were higher than those obtained in HS medium, independently of culture conditions. The highest yields obtained in P culture medium could be related to the higher C content when compared with HS. Therefore, pineapple waste could be considered as a potential carbon rich source.

On the other hand, BC yields from the dynamic culture were lower than that obtained from static conditions.[31] The gelatinous cellulose membrane in the static culture grows on the interface between the air (rich in oxygen) and the liquid medium, whereas sphere-like particles are developed suspended in the liquid medium. Therefore, particles only have access to the oxygen dissolved in the medium. Nonetheless, the optimization process of the dynamic culture could be envisaged using an aerated fermentation system.[32] In this way, it is important to develop not only cellulose membranes, but also other cellulose production forms, in order to increase the potential applications of these unique materials.[6,8]

As described in the experimental part, the PD was determined by means of viscosimetry. In this work, PD values between 2600 and 3000 were obtained in all cases (Table I). As it can be noted, BC produced in D culture conditions had a lower PD compared with that produced in S conditions. The results are in accordance with the study of Watanabe et al.[23] According to other authors, different PD values were obtained, usually between 2000 and 6000,[33] but in some cases reaching even values of 10.000 or 14.000 depending on the culture conditions.[24,33]

As already mentioned, cellulose membranes or sphere-like particles were produced by microorganisms in the form of swollen gel with 98% of water. The hidrophillicity depends also on the interstitial spaces of the inner area in the never dried matrix. Due to its hydrophilic nature, the WHC of BC is an important feature in a view of potential applications.[6] Figure 1 shows the WHC calculated after rehydration of BC cultivated in different conditions. As can be observed, higher WHC was achieved in standard medium. In both media, the WHC was slightly higher in dynamic conditions. This behavior might be related with the cellulose membrane and particles inner morphology, structure regularity and free space or pores of the 3D networks of the nanofibers formed during fermentation. This fact will be analyzed in the morphological characterization by SEM.

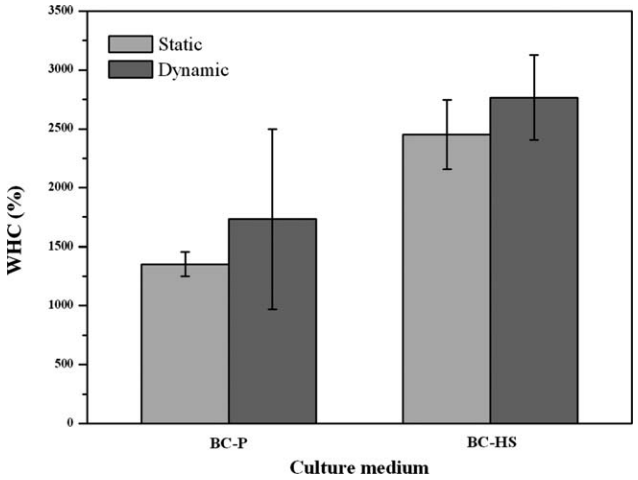

Figure 1. WHC of BC produced in P and HS media in static and dynamic conditions.

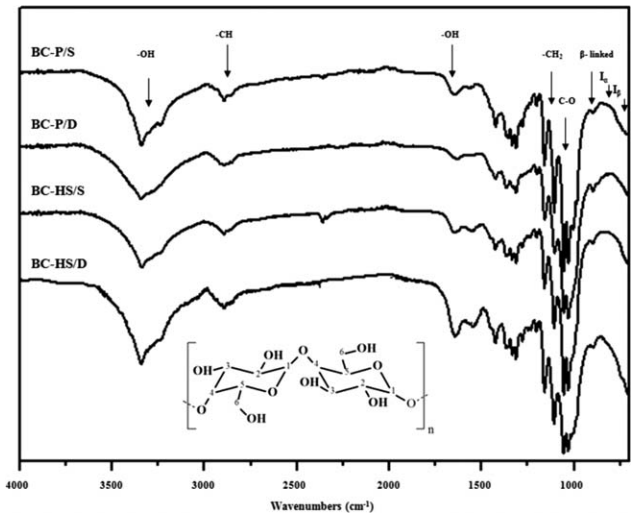

Figure 2. ATR-FTIR spectra of BC in static and dynamic culture conditions.

The chemical structure of different BC samples was studied by ATR-FTIR. The spectra of the biosynthesized BC samples are shown in Figure 2. The spectra in all cases shows the characteristic adsorption bands of BC functional groups showing the typical cellulose I pattern reported by other authors.[34–36] The bands located at around 3300 cm^{-1} correspond to O—H cellulose stretching vibrations. The absorption bands at 2900–2880 cm^{-1} and 1460–1250 cm^{-1} are assigned to the CH and CH_2 stretching and bending vibrations, respectively. Furthermore, the bands at 1170–1050 cm^{-1} are assigned to the vibrations of the C—O—C bond of the glycosidic bridges. The broad band at 897 cm^{-1} is characteristic of β-linked glucose based polymers. Finally, the band at around 1650 cm^{-1} is assigned to the water absorbed by the cellulose.

The chemical structure of BC samples in different media and conditions was also studied by ^{13}C CP/MAS spectra as it is shown in Figure 3. Peaks at 105, 88, and 64 ppm correspond to C1, C4, and C6 carbons, respectively. The signals from C2, C3, and C5 atoms are in the range of 70–80 ppm. As expected, all BC samples showed the typical spectrum of the cellulose in the 110–60 ppm region. The results were similar to those obtained by Yamamoto and Horii (1994) and by Castro et al. (2012) with the same genus of bacteria.[24,37]

The results of the chemical characterization of BC samples have confirmed the same chemical nature and high purity for all biosynthesized samples independent of the media and fermentation conditions.

Figure 4 shows X-ray diffraction patterns of the different BC samples. The patterns present the typical crystalline structure of native cellulose, i.e., three main peaks located at $2\theta = 14.5°$, 16.8°, and 22.7° that correspond to the primary diffraction of the (1–10), (110), and (200) planes of cellulose I polymorph.[38,39] The values of CI, calculated from eq. (3), are listed in Table I. In general, based on these preliminary values, it can be observed that cellulose produced in dynamic conditions presented a slight reduction in the CI compared with those obtained in static conditions, as previously reported by Iwata et al.[40] Similarly, Guo et al. (2012b) found that smaller elementary fibril crystals and more amorphous regions were formed under dynamic conditions and, therefore, lower crystallinity values were obtained.[41] The agitation of dynamic condition might generate stresses that affect the packing of cellulose chains, which can affect to the overall BC crystallity.

The morphology of the BC sphere-like particles and membranes were analyzed by digital photographs and SEM images. Figure 5 depicts digital photographs of BC membrane and sphere-like particles obtained using static and dynamic cultures, respectively. In static culture, cellulose was formed on the surface

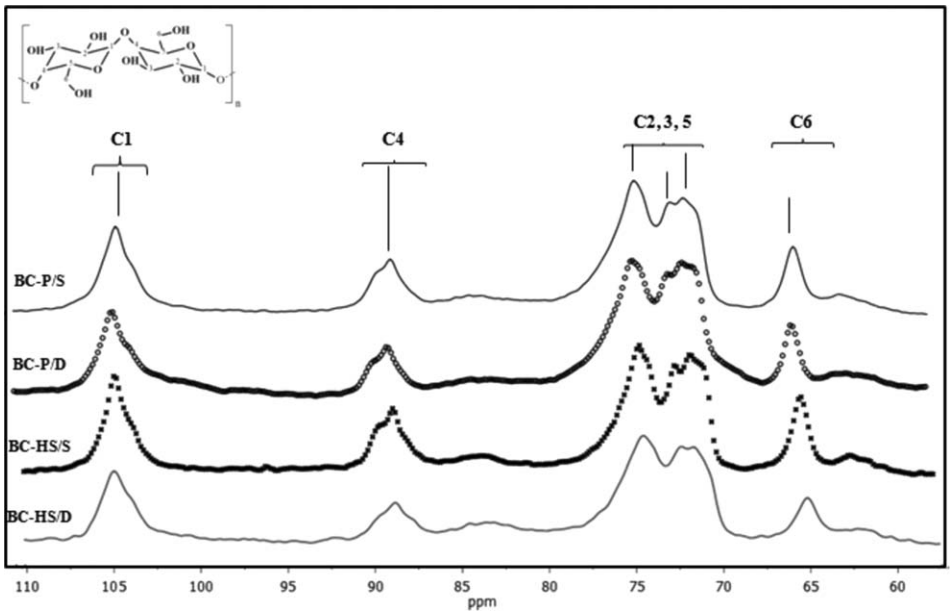

Figure 3. ^{13}C CP/MAS NMR spectra of cellulose produced in different culture conditions.

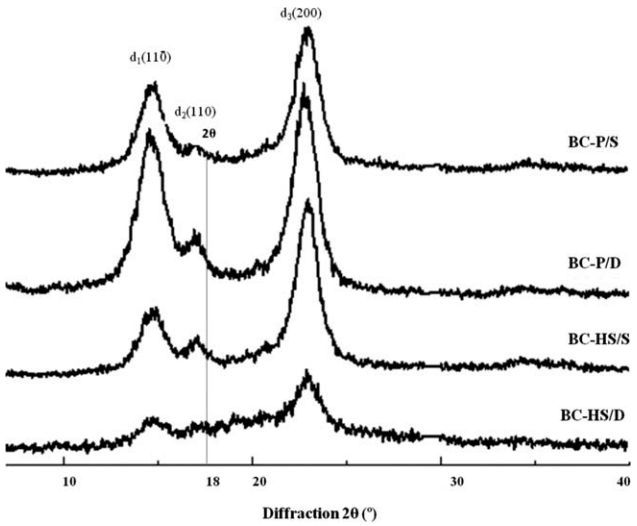

Figure 4. X-ray diffraction patterns of BC produced in different culture conditions.

resulting in a gelatinous membrane, with a thickness of 0.5–1 cm for both culture media. Meanwhile, under dynamic conditions, BC was obtained in the form of sphere-like particles with a diameter between 0.2 and 0.5 cm in the P medium, and between 1 and 3 cm in the case of HS medium. Previous studies reported BC sphere diameters of 0.6–0.8 cm under dynamic conditions.[41] The size of the pellets depends strongly on the culture medium.[22,41,43] The difference in diameter and number of the sphere-like particles of both culture media can be due to the composition of the medium. P medium is richer in carbon, so it seems that the probability to form more nuclei is higher when higher carbon content is. Since the nucleation phenomenon prevails over the growing process, there is lower space for the microfibrils to grow leading to spheres with lower size.

The surface morphology of the three-dimensional network structure of different BC is shown in Figure 6. Considering the dimensions of microfibrils in static conditions, no noticeable difference in diameter was observed between BC synthesized in P and HS culture with diameters of 30–40 nm, approximately. Slightly higher microfibril diameters were measured in both media in dynamic conditions, between 40 and 60 nm, approximately. Ruka et al. (2012) reported an approximate diameter of 24–55 nm for BC microfibrils produced under dynamic state depending on the culture conditions.[41] In this figure, the surface of BC (BC-P/S) membrane and (BC-HS/D) sphere-like particles are compared, due those samples presented more marked differences in properties as shown in Table I. These images reveal morphological differences between static and dynamic conditions. As can be observed, areas with higher porosity were obtained under D conditions. In dynamic conditions, due to agitation, tensions between BC microfibrils were produced. Furthermore, higher stresses may hinder the packing process, resulting in fibrils with smaller CI as observed by XRD analysis. SEM micrographs also reveal that the voids between cellulose ribbons in both BC-P/S and BC-HS/S samples decreased,

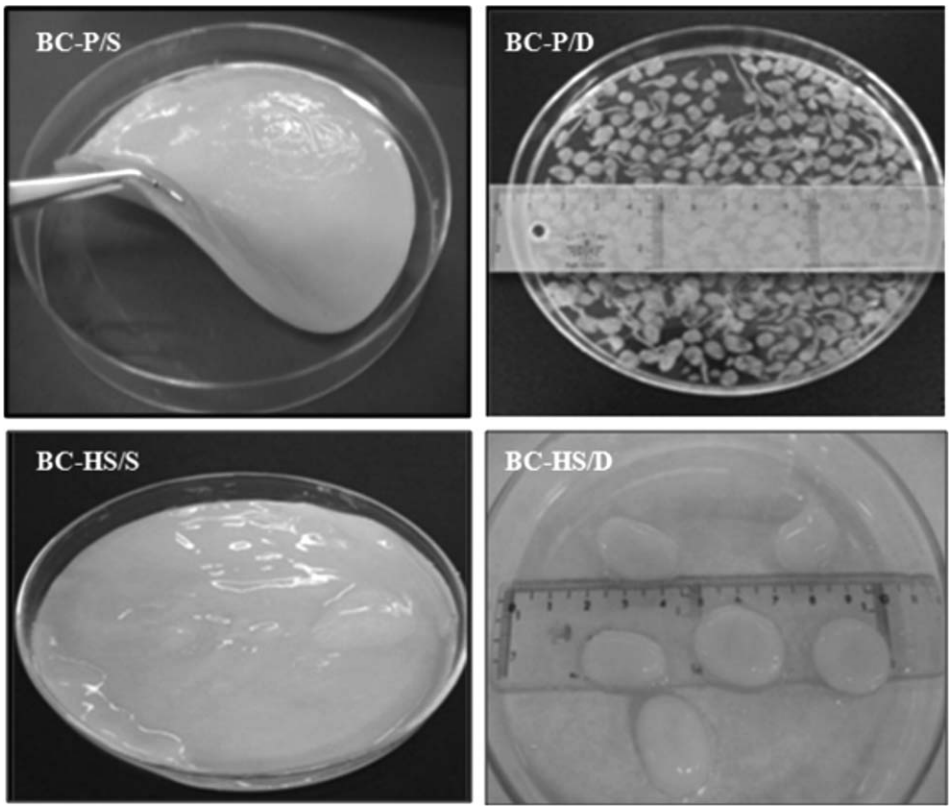

Figure 5. Digital images of BC samples produced in static and dynamic cultures using two different sources.

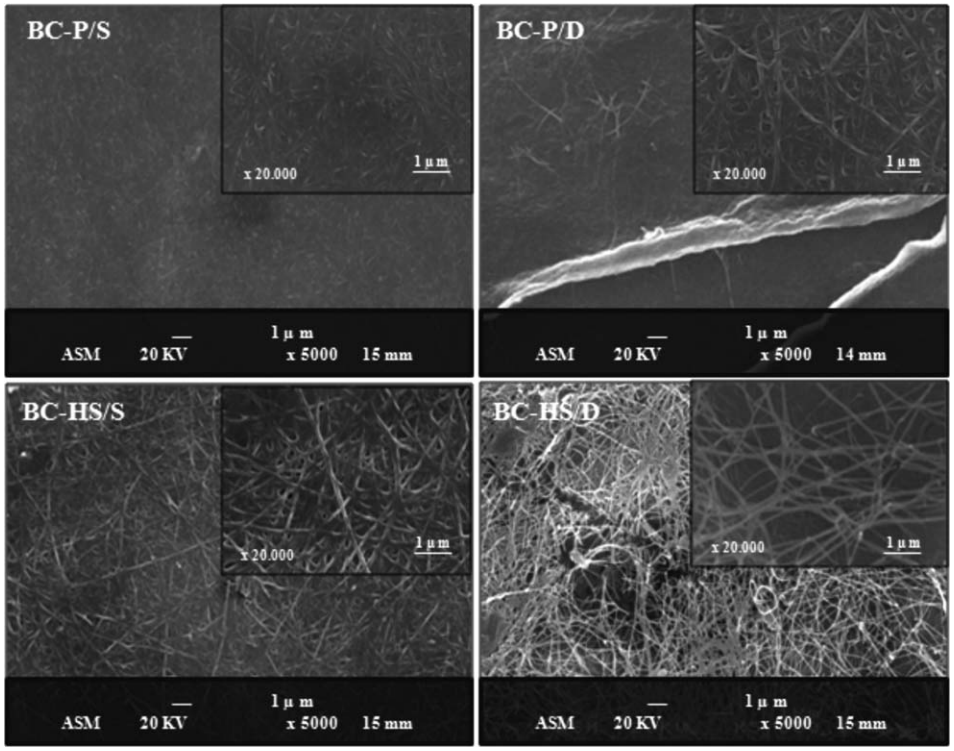

Figure 6. SEM images of the surface layer of BC produced in static and dynamic conditions.

compared to those obtained under D condition, resulting in a denser cellulose network and, subsequently with less free space to absorb water, agreeing whit WHC results.

Figure 7 shows the images of the cross section area of BC obtained in the S and D culture conditions. Cellulose synthesized under dynamic conditions, showed a more disordered structure than cellulose synthesized under static conditions.[4] In Figure 8, a magnified image of the internal structure of BC is shown for comparison. As can be observed, the BC membranes produced in static conditions exhibits an ultrafine network with

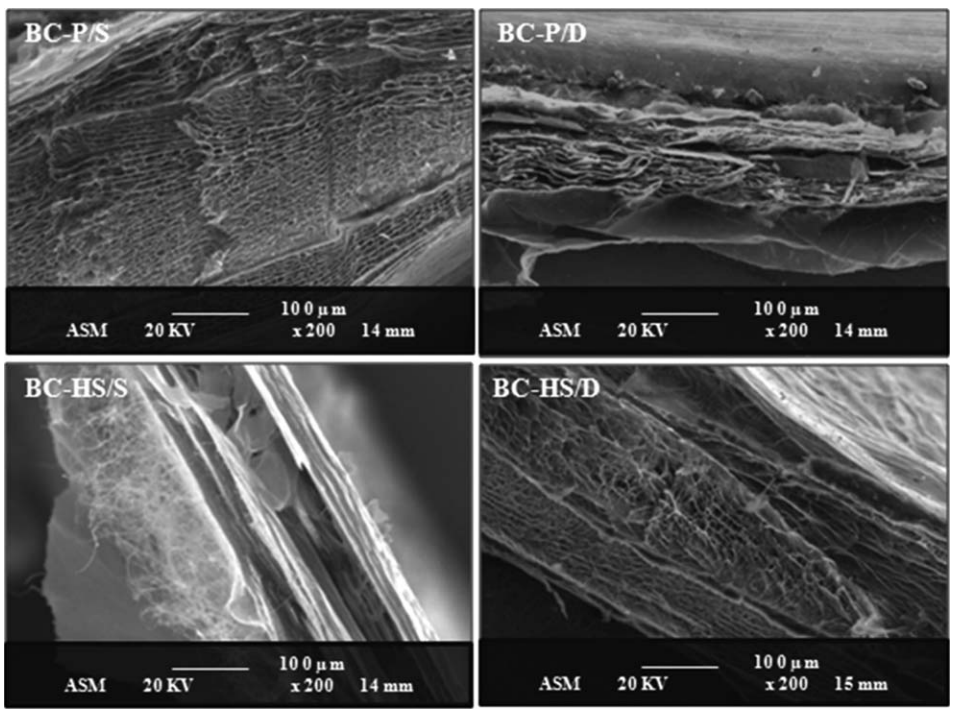

Figure 7. SEM images of BC cross-section produced in different culture media and conditions.

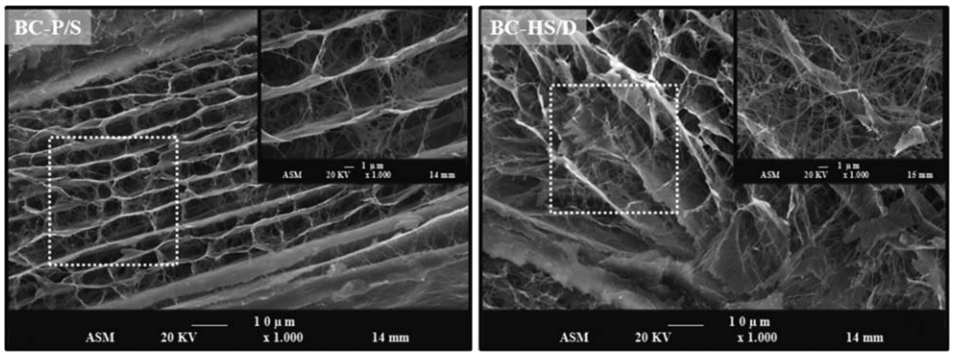

Figure 8. SEM images of BC cross-section in both culture conditions.

a well-organized hierarchical structure formed with different layers separated by a distance of 0.5–0.6 µm, approximately. In contrast, in the case of BC-HS/D, the internal structures didn't present a well-organized structure.

CONCLUSIONS

This study intends to produce BC with different morphologies (membrane and sphere-like) using nonconventional culture, i.e., using sugar cane juice and pineapple residues, both in static and dynamic conditions. The yield of BC was higher than those obtained with the respective standard media. However, the physico-chemical properties studied by ATR-FTIR and NMR of BC produced from P medium were similar than those obtained from standard culture, regardless of the culture conditions employed. Even so, the morphology of BC samples appears to be affected by the use of different media and conditions. In dynamic conditions, due to the biosynthesis process conditions the generated 3D structure resulted in a lower crystallinity, PD, and yield. In addition, the formed structure showed high porosity and, consequently, large WHC. These results strongly suggest that not only the production of BC under nonconventional (pineapple waste) medium was possible, but also BC with different morphologies using low cost nutrient residues could be produced contributing to the production of high value products and proper waste management.

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REFERENCES

1. Brown, A. J. J. *Chem. Soc.* **1886a**, *49*, 432.
2. Brown, A. J. J. *Chem. Soc.* **1886b**, *49*, 172.
3. Yan, Z.; Chen, S.; Wang, H.; Wang, B.; Wang, C.; Jiang, J. *Carbohydr. Res.* **2008**, *343*, 73.
4. Czaja, W.; Romanovicz, D.; Brown, R. M. *Cellulose* **2004**, *11*, 403.
5. Chawla, P. R.; Bajaj, I. B.; Shrikant, A. S.; Singhal, R. S. *Food Technol. Biotechnol.* **2009**, *47*, 107.
6. Klemm, D.; Schumann, D.; Udhardt, U.; Marsch, S. *Prog. Polym. Sci.* **2001**, *26*, 1561.
7. Helenius, G.; Bacjdahi, H.; Bodin, A.; Nannmark, U.; Gatenholm, P.; Risberg, B. *J. Biomed. Mater. Res.* **2006**, *76*, 431.
8. Czaja, W.; Krystynowicz, A.; Bielecki, S.; Brown, R. M. *Biomaterials* **2006**, *27*, 145.
9. Bae, S.; Shoda, M. *Appl. Microbiol. Biotech.* **2005a**, *67*, 45.
10. Bae, S; Shoda, M. *J. Biosci. Bioeng.* **2005b**, *97*, 33.
11. Keshk, S.; Sameshima, K. *Afr. J. Biotechnol.* **2006**, *4*, 478.
12. Goelzer, F. D. E.; Faria-Tischer, P. C. S.; Vitorino, J. C.; Sierakowaki, M. R.; Tischer, C. A. *Mater. Sci. Eng.* **2009**, *29*, 546.
13. Kurosumi, A.; Sasaki, C.; Yamashita, Y.; Nakamura, Y. *Carbohydr. Polym.* **2009**, *76*, 333.
14. Shigematsu, T.; Takamine, K.; Kitazato, M.; Naritomi, T.; Morimura, S.; Kida, K., *J. Biosci. Bioeng.* **2005**, *99*, 415.
15. Hong, F.; Qui, K. *Carbohydr. Polym.* **2008**, *72*, 545.
16. Carreira, P.; Mendes, J. A. S.; Trovatti, E.; Serafin, S. L.; Freire, C.S.R.; Silvestre, A. J. D.; Neto, C. P. *Bioresour. Technol.* **2011**, *102*, 7354.
17. Chen, L.; Hong, F.; Yang, X.; Han, S. *Bioresour. Technol.* **2013**, *135*, 464.
18. Gomes, F. P.; Silva, N. H. C.S.; Trovatti, E. Serafim, L. S.; Duarte, M. F.; Silvestre, A. J. D.; Neto, C. P.; Freire, S. R. C. *Biom. Bioener.* **2013**, 205.
19. Sasaki, M.; Jojima, T.; Kawaguchi, H.; Inui, M.; Yukawa, H. *Appl. Microbiol. Biotechnol.* **2009**, *85*, 105.
20. Chao, Y.; Ishida, Y.; Sugano, T.; Shoda, K. *Biotechnol. Bioeng.* **2000**, *68*, 345.
21. Guo, J.; Catchmark, J. M. *Carbohydr. Polym.* **2002a**, *87*, 1026.
22. Hu, Y.; Catchmark, J. M. *Biomacromolecules* **2010**, *11*, 1727.
23. Zan, Z.; Chen, S.;Wang, H.; Wang, B.; Wang, C.; Jiang, J. *Carbohydr. Res.* **2008**, *343*, 73.
24. Watanabe, K.; Tabuchi, M.; Morinaga, Y.; Yoshinaga, F. *Cellulose* **1998**, *5*, 187.

25. Castro, C.; Zuluaga, R.; Álvarez, C.; Putaux, J. L.; Caroa, G.; Rojas, O. J.; Mondragon, I. *Carbohydr. Polym.* **2012**, *89*, 1033.
26. Retegi, A.; Gailondo, N.; Peña, C.; Zuluaga, R.; Castro, C.; Gañan, P.; de la caba, K.; Mondragon, I. *Cellulose* **2010**, *17*, 661.
27. Hestrin, S.; Scharmm, M. *Biochem. J.* **1954**, *58*, 345.
28. ASTM- American Society for Testing and Materials. Test Method for Intrinsic Viscosity of Cellulose, ASTM- D1795, doi: 10.1520/D1795-96R07E01.
29. Marx-Figini, M. *Polym. Bull.* **1987**, *17*, 225.
30. Segal, L.; Creely, J. J.; Martin, A. E. J.; Conrad, C. M. *Textile Res. J.* **1959**, *29*, 786.
31. Yoshinaga, F.; Tonouchi, N.; Watanabe, K.; Yoshinaga, N. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 219.
32. Cheng, H. P; Wang, P. M.; Chen, J. W.; Wu, W. T. *Biotechnol. Appl. Biochem.* **2002**, *35*, 125.
33. Jonas, R.; Farad, L. F. *Polym. Degrad. Stab.* **1998**, *59*, 101.
34. Huang, H. C.; Chen, C. L.; Bin, L. S.; Ping, H. C.; Huang, C. H. *Bioresour. Technol.* **2010**, *101*, 6084.
35. Yamamoto, H.; Horii, F.; Hirai, A. *Cellulose* **1993**, *3*, 229.
36. Nelson, M. L.; O'Connor, R. T. *Appl. Polym. Sci.* **1964**, *8*, 1325.
37. Yamamoto, H.; Horii, F. *Cellulose* **1994**, *1*, 57.
38. Klechkovskaya, V. V.; Baklagina, N. D.; Stepine, N. D.; Khripunov, A.; Buffat, P. A.; Suvorova, E. I.; Zanaveskina, I. S.; Tkchenko, A. A.; Gladchenko, S. V. *Crystallogr. Rep.* **2003**, *48*, 755.
39. Zugenmaier, P. *Prog. Polym. Sci.* **2001**, *26*, 1341.
40. Iwata, T.; Indrarti, L.; Azuma, J. I. *Cellulose* **1998**, *5*, 215.
41. Guo, J.; Catchmark, J. M. *Carbohydr. Polym.* **2012b**, *88*, 547.
42. Ruka, D. R; Simon, G. P.; Dean, M. K. *Carbohydr. Polym.* **2012**, *89*, 613.
43. Jin, G.; Jeffrey, M. C. *Carbohydr. Polym.* **2012**, *88*, 547.