CHEMICAL MODIFICATION OF BACTERIAL CELLULOSE

FOR USE IN REGENERATIVE MEDICINE

JORGE E. RODRIGUEZ-CHANFRAU, MÁRCIO LUIZ DOS SANTOS,[*] CARLA DOS SANTOS RICCARDI, GABRIEL MOLINA DE OLYVEIRA, MARGARITA HERNÁNDEZ-ESCALONA,[**] PIERRE BASMAJI,[***] YAYMARILIS VERANES-PANTOJA[**] and ANTONIO CARLOS GUASTALDI

Department of Physical Chemistry, Institute of Chemistry, Campus Araraquara, Paulista State University "Júlio de Mesquita Filho", São Paulo, 14800-060, Brazil
[*]*Federal University of ABC – UFABC, Center of Natural and Human Sciences, 09210-580 Santo André, São Paulo, Brazil*
[**]*Center of Biomaterials, University of Havana, Ave. Universidad s/n, e/G y Ronda, Vedado, La Habana, CP 10400, Cuba*
[***]*Innovatec's – Biotechnology Research and Development, São Carlos-SP, 13560-042, Brazil*
✉ *Corresponding author: Jorge E. Rodríguez-Chanfrau, jerodriguez354@gmail.com*

Biomaterials are suitable for treating or relieving the symptoms of certain diseases or injuries, and they are also used for replacing damaged tissue or organs and modifying a patient's anatomy or physiological process. Currently, biomaterials are an important part of the medical industry. Bacterial cellulose is a biomaterial with great potential in several applications due to its characteristics and high purity. These characteristics allow its application in the confection of scaffolds for tissue regeneration, medical applications and nanocomposites. In this work, the bacterial cellulose was modified by acid treatment with sulfuric acid. The effect of sulfuric acid on bacterial cellulose was analyzed using thermogravimetric analysis, differential scanning calorimetry, X-ray powder diffraction, FTIR spectroscopy and scanning electron microscopy. The results showed that the solubility of the initial sample increased by approximately 18%, while the yield exceeded 80%. On the other hand, a decrease of the crystallinity index, which facilitated solubility, was observed. The increase of the sulfuric acid concentration favored this process and, as a result, a material with a different morphological surface was obtained.

Keywords: bacterial cellulose, crystallinity index, infrared spectroscopy, X-ray diffraction, hydrogen bond intensity, lateral order index

INTRODUCTION

Due to a number of considerations, such as the aging of the population, the increase of life expectancy, an increasing demand for a good quality of life, and, on the other hand, the high rates of traffic accidents, as well as many economic and technological aspects, researchers felt the need to develop a new generation of biomaterials for tissue regeneration. Due to the great importance of these materials, their demand has been increasing each day. Degenerative diseases represent a significant proportion of chronic, progressive and often fatal diseases, which are associated with a progressive decline in tissue functions that share many hallmarks of aging. Such diseases incur profound human and social costs, and no effective therapeutical approaches to them have been found up to the present.[1]

There are 600 million people in the world aged 60 year old or over, and this will double by 2025, reaching 2 billion by 2050. The economic impact of morbidity in elderly population represents a significant burden, which requires effective and rapid solutions.[1]

Biomaterials are an important part of the medical device industry, and currently they are becoming more prevalent as scaffolds in the development of sophisticated therapeutical products, such as sustained release drug delivery systems. However, the design of new biomaterials

is still a challenge, as it is currently not possible to freely choose among components that should be assembled/connected to each other. The development of design principles for new materials is based on understanding and quantification of the relationship among scaffold characteristics, such as molecular composition, morphology and physical properties and the *in vivo* outcome, all these are still a challenge for the science at the present time.[1]

Cellulose is a semicrystalline polymer and its crystallinity depends on the source of isolation and processing methods. The complex structural hierarchy of cellulose, due to its profuse hydrogen bonding, is manifested by the existence of several polymorphs (crystalline forms). Native cellulose has a polymorph structure, which exists in two crystalline forms: Iá (in algae and bacteria) and Iâ (in higher plants).[2,3]

Although it is chemically identical to plant cellulose, the cellulose synthesized by bacteria has a fibrillar nanostructure, which determines its physical and mechanical properties – characteristics that are necessary for modern medicine and biomedical research.[4,5]

Cellulose is renewable, biodegradable and biocompatible and it can be derivatized to yield various useful products. Yet, cellulose has poor solubility because of the high amount of hydrogen bonds present in its molecule.[6] This phenomenon limits the application of cellulose in the development of biomaterials for medical use. This disadvantage can be, however, overcome, and this is conventionally done by chemical modification of the cellulose. The objective of this work was to chemically modify bacterial cellulose through an acid treatment to increase its potential application in regenerative medicine.

EXPERIMENTAL

Bacterial cellulose membranes were supplied by Innovatecs Products Biotechnological LTDA, São Carlos, São Paulo, Brazil. The acetic fermentation process was achieved using glucose as a carbohydrate source. The results of this process are vinegar and a nanobiocellulose biomass. Bacterial cellulose (BC) was produced by Gram-negative bacteria *Gluconacetobacter xylinus*, and could be obtained from the culture medium as a pure 3-D structure, consisting of an ultra-fine network of cellulose nanofibers.[7-9]

To achieve the modification of the cellulose, sulfuric acid (48% and 64%) was used.[10] Sulfuric acid was supplied by Merck. The cellulose fibers were hydrolyzed in acid medium at room temperature (32 ±

2 ºC) under constant stirring for 60 min. In both cases, the hydrolyzed pulp was thoroughly washed with distilled water until pH 7.0 was reached, and then it was wetted with ethanol and dried in an oven at 37 °C to a constant mass.

The yield was determined from the regenerated cellulose on the basis of the initial weighing, according to the following equation:[11]

$$Y\,(\%) = (w/w_o)\,100 \qquad (1)$$

where w_o is the initial dry weight of the sample and w is the weight of the dried sediment.

Also, the solubility (S) of cellulose in the solutions of sulfuric acid was calculated as follows:

$$S\,(\%) = 100\,[1 - (w/w_o)] \qquad (2)$$

where w_o is the initial dry weight of the sample and w is the dry weight of the insoluble part of the sample.

Differential scanning calorimetry
Differential scanning calorimetry (DSC) thermograms of the untreated and treated bacterial celluloses were obtained by using a TA Instruments DSC Q100 differential scanning calorimeter (USA). The samples were accurately weighed in aluminum pans and sealed. According to this method, a small hole was made at the top of the pan in order to allow the release of the moisture. A nitrogen purge, with a flow rate of 50 mL/min, was used in the furnace. The measurements were performed at a heating rate of 5 ºC/min from 0 to 300 ºC.

Thermogravimetric analysis
The thermal stability of the cellulose extract was determined using TA Instruments SDT-2960 Simultaneous DTA/DTG equipment (USA). The analysis was performed on samples of 10-15 mg in a nitrogen atmosphere from 30 ºC to 800 ºC at a heating rate of 5 °C/minute.

X-ray powder diffraction studies
XRD spectra were recorded at room temperature (25 ºC) with a SIEMENS D5000, DIFFRAC PLUS XRD diffractometer (Germany) with BRAGG-Brentano geometry, Cu Kα radiation (λ = 0.154 nm), a Flicker detector and a graphite monochromator. The scattering angle range from 4º to 80º with a 2θ step interval of 0.02º was used. Cellulose samples were cut into small pieces, laid on a glass sample holder, and analyzed under plateau conditions. An operating voltage of 40 kV and current of 30 mA were utilized, and the intensities were measured in the range of 5° < 2θ < 30°. Peak separations were carried out using Gaussian deconvolution. The d-spacings were calculated using the Bragg equation. Crystallographic search match software was used to identify the crystal structure of the samples.

The surface method for estimating the crystallinity index of the cellulose samples was carried out

according to Ciolacu *et al.*,[12] using the following equation:

CrI (%) = (*Sc/St*) · 100

where *Sc* is the area of the crystalline domain and *St* is the area of the total domain.

The apparent crystallite size (*L*) was determined using the Scherrer equation; the surface chains occupied a layer of approximately 0.57 nm thickness, so the proportion of the crystallite interior chains (X) was calculated according to Poletto *et al.*[13]

The Z-discriminant function developed by Wada and Okano[14] was calculated using the following equation:

$Z = 1693d_1 - 902d_2 - 594$

where d_1 is the d-spacing of the peak ($\overline{1}$10); d_2 is the d-spacing of the peak (110); and $Z > 0$ indicates Iα; while $Z < 0$ indicates the Iβ dominant type.

FTIR spectroscopy

The FTIR spectra of the bacterial cellulose samples were recorded on a FTIR VERTEX 70/BRUKER spectrometer (Germany). A total of 64 cumulative scans were taken, with a resolution of 4 cm^{-1}, in the frequency range of 4000 to 400 cm^{-1}, in the transmission mode. The HBI (hydrogen bond intensity), LOI (lateral order index) and cellulose I/cellulose II ratio were determined.[15,16]

Scanning electron microscopy

The scanning electron microscopy (SEM) imaging of crystalline cellulose was carried out using a FEG-MEV; JEOL 7500F scanning electron microscope (Germany). The equipment was operated at an acceleration voltage of 2 kV. For each sample, different parts of the grid were used to determine both average shape and size distributions. The samples were coated with a carbon layer with a thickness of 15 nm.

RESULTS AND DISCUSSION

The effect of the sulfuric acid concentration on the structure and the properties of the cellulose were studied. Table 1 shows that the solubility of the initial sample increased by approximately 18%, while the yield exceeded 80%. These results

indicate that the treatment performed did not affect the yield under these working conditions.

The TG curves for all the samples exhibited two stages of mass loss within the temperature range 25-600 °C (Fig. 1a, 1b and 1c). The first degradation occurred around 51 °C for all the samples, which was assigned to water evaporation.[17-20] For the bacterial cellulose sample, the second degradation stage was observed between 100 °C and 350 °C, incurring a mass loss (Fig. 1). In this range, two peaks of degradation were observed (108 °C and 180.7 °C). Kumar *et al.*[20] reported that intermolecular H-bonded water evaporated around 120 °C. This result suggests that the peak observed at 108 °C may be related to the loss of intermolecular water of the sample evaluated, while the peak noted at 180.7 °C is linked to the thermal degradation of cellulose (Fig. 1a).

In the case of the samples treated with sulfuric acid, the TG curve showed two peaks of degradation, where the lower temperature peak may correspond to the sulfated amorphous region, whereas the higher temperature peak was assigned to the unsulfured part of the material. It was also observed that upon increasing the concentration of sulfuric acid, the degradation peak corresponding to the unsulfured part of the material was shifted to a lower temperature (327.9 °C and 256 °C for 48% and 64%, respectively) (Fig. 1b and 1c).

The DSC curves exhibit a light endothermic peak, characteristic of cellulose, in the 50-150 ºC region, which corresponds to the dehydration process (Fig. 1d). In the samples treated with different concentrations of sulfuric acid, a shift of maximum temperature of the dehydration process to lower values may be observed with an increment of the sulfuric acid concentration (104 °C and 97.5 °C for 48% and 64%, respectively).

Table 1
Crystallinity index, yield and solubility of samples treated with sulfuric acid

Treated sample	CI (%)	Yield (%)	Solubility (%)
H$_2$SO$_4$ 48%	60.1	82.3	17.7
H$_2$SO$_4$ 64%	54.7	81.8	18.2
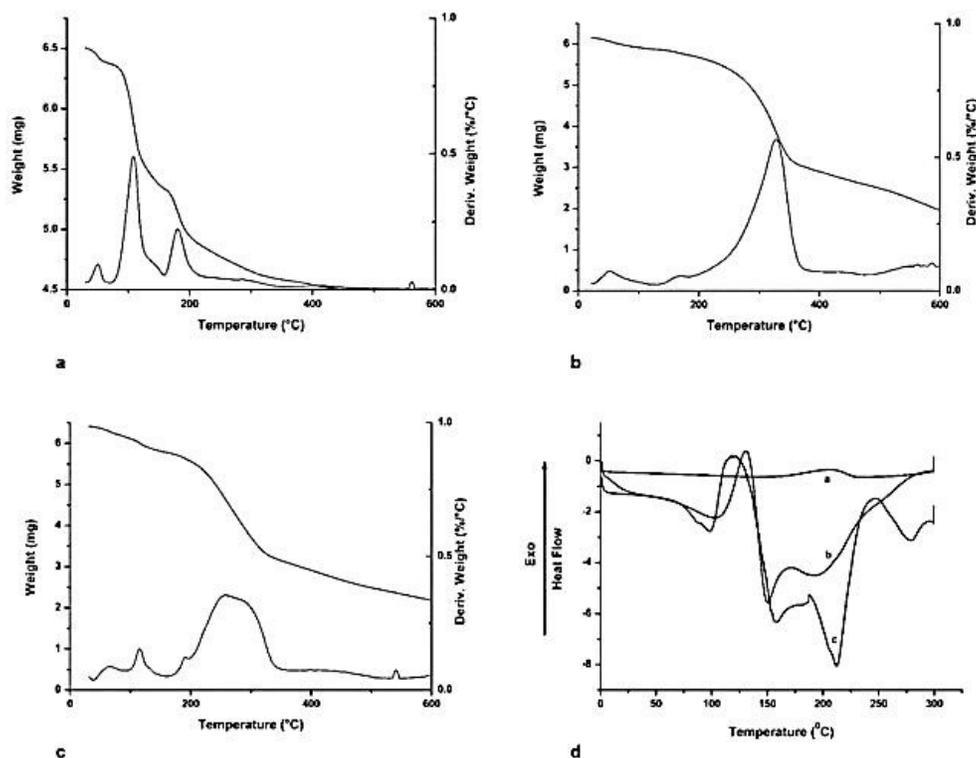
Figure 1: TG analysis of bacterial cellulose (a), bacterial cellulose treated with 48% sulfuric acid (b); and bacterial cellulose treated with 64% sulfuric acid (c); as well as DSC analysis (d) (a: bacterial cellulose, b: bacterial cellulose treated with 48% sulfuric acid and c: bacterial cellulose treated with 64% sulfuric acid)

In the region between 150 and 250 ºC, the samples treated with sulfuric acid present two endothermic peaks (150.2 °C and 191.5 °C for the samples treated with 48% sulfuric acid, and 156.8 °C and 211.7 °C for the samples treated with 64% sulfuric acid). Meanwhile, in the case of the sample treated with the highest concentration of sulfuric acid, an endothermic peak at 279.2 ºC was observed (Fig. 1d).

The XRD of untreated bacterial cellulose shows three diffraction peaks at $2\theta = 16.6°$; 22.7° and 35.3°, which are attributed to bacterial cellulose I (crystalline structure).[20-22] Bacterial cellulose was identified as a native cellulose (PDF 502241) and its characteristic peaks are indexed. These peaks correspond to the (110), (200), and (004) diffraction planes, respectively.[21,23]

The XRD patterns of different samples are illustrated in Figure 2. A decrease is observed in the intensities of the diffractograms for both treated samples, compared with the untreated cellulose. On the other hand, a low intensity peak is observed at $2\theta = 11°$ in the sample treated with 48% sulfuric acid. According to literature, this diffraction peak is representative of the cellulose-

III polymorph.[24] In the sample treated with 64% sulfuric acid, this peak was not observed.

The band position (2θ values) and d-spacings of the celluloses, calculated from X-ray diffractogram profiles, are illustrated in Table 2. The values of band position and d-spacings were similar.

The crystallinity index of untreated cellulose was 73.9%. The calculated crystallinity indexes of the differently treated samples are given in Table 1. A strong decrease of the crystallinity degree to values of around 19% and 25%, for the samples treated with 48% or 64% sulfuric acid solutions, respectively, was observed. This fact can be explained by a reduction in the intra- and inter-molecular hydrogen bonds, occurring during the hydrolysis process.

The proportion of crystallite interior chains shows slight differences between the treated samples and the untreated one. On the other hand, the Z-values for the treated samples indicate that the cellulose samples belong to the Iβ dominant type (Z<0), while the untreated sample belongs to the dominant type Iα (Z>0) (Table 3).

676
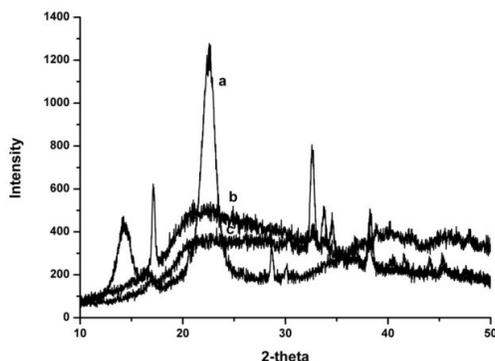
Figure 2: X-ray diffractograms of a: bacterial cellulose, b: bacterial cellulose treated with 48% sulfuric acid, and c: bacterial cellulose treated with 64% sulfuric acid

Table 2
Band position (2θ) and d-spacings of crystalline and amorphous cellulose regions for the samples studied

Samples	($\bar{1}$10)		(110)		Amorphous	(200)	
	2θ	d (nm)	2θ	d (nm)	2θ	2θ	d (nm)
CB	14.2	0.6216	16.6	0.5359	20.6	22.7	0.3999
H_2SO_4 48%	14.9	0.5926	17.0	0.5138	20.2	23.1	0.3799
H_2SO_4 64%	14.4	0.6143	17.0	0.5885	20.3	22.8	0.3919

Table 3
Parameters obtained from the XRD analysis of the samples studied

Samples	L 200 (nm)	X	Z
CB	2.99	0.3828	19.99
H_2SO_4 48%	2.82	0.3549	- 9.18
H_2SO_4 64%	2.92	0.3716	- 39.82
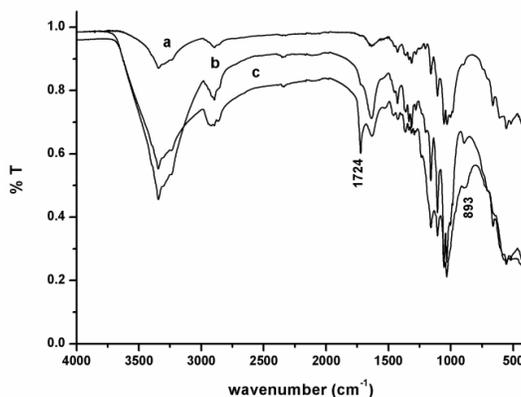
Figure 3: FITR analysis of initial bacterial cellulose (a), bacterial cellulose treated with 48% sulfuric acid (b) and bacterial cellulose treated with 64% sulfuric acid (c)

Figure 3 shows the FTIR spectra of untreated and treated bacterial cellulose. The bands at 3341 cm^{-1} (O-H stretching of intra- and intermolecular H-bonds for cellulose I), 2892 cm^{-1} (C-H stretching), 1635 cm^{-1} (associated to the bending mode of the naturally absorbed water), as well as those at 1425, 1323, 1163, 1163, 1036, and 894 cm^{-1}, are associated with bacterial cellulose.[5,21,24,25] After the treatment with sulfuric

acid, a band at 1724 cm^{-1} (C-O stretching vibration for the ester linkages) was observed. On the other hand, a sharper band at 893 cm^{-1} associated with amorphous cellulose was also remarked. Both bands were more intense in the cellulose treated with 64% sulfuric acid. Due to the absorption of water, the peak at 1635 cm^{-1} intensifies. The parameters HBI and LOI were calculated. HBI decreased by around 30% in the samples of cellulose treated with sulfuric acid. Also, LOI and cellulose I/cellulose II ratio decreased with the sulfuric acid treatment of the cellulose (Table 4).

Figure 4 shows SEM micrographs of the untreated cellulose and of the cellulose treated with different concentrations of sulfuric acid. It may be observed that the untreated bacterial cellulose shows a three-dimensional structure formed by nanometric fibers (Fig. 4a). In the case of the samples treated with sulfuric acid, a swelling of the fiber may be noted (Fig. 4b and 4c).

The degradation stage that the bacterial cellulose sample underwent was due to the degradation processes of cellulose, such as depolymerization, dehydration, and decomposition of glycosyl units, followed by the formation of a charred residue.[17-20]

The difference in the thermal stability of the samples can be attributed to variations in the crystallinity, moisture content, porous structure, and polymerization degree of the materials before and after the chemical treatment. It has been reported in the literature that, in samples hydrolyzed with sulfuric acid, an increase in sulfate ions concentration leads to a decrease in the degradation temperature of the cellulose prepared. This may be explained by the porosity of the structure and the ability of bacterial cellulose to absorb water and promote swelling.[26] On the other hand, sulfuric acid is a dehydrating agent, which facilitates the decomposition of the cellulose by removing some of the –OH groups by a mechanism of esterification.[21,27,28]

Table 4
FTIR analysis parameters for calculated HBI, LOI and cellulose I/cellulose II ratio

Samples	HBI	LOI	Cellulose I/cellulose II
CB	0.98	0.96	0.83
H$_2$SO$_4$ 48%	0.66	0.84	0.51
H$_2$SO$_4$ 64%	0.79	0.71	0.62
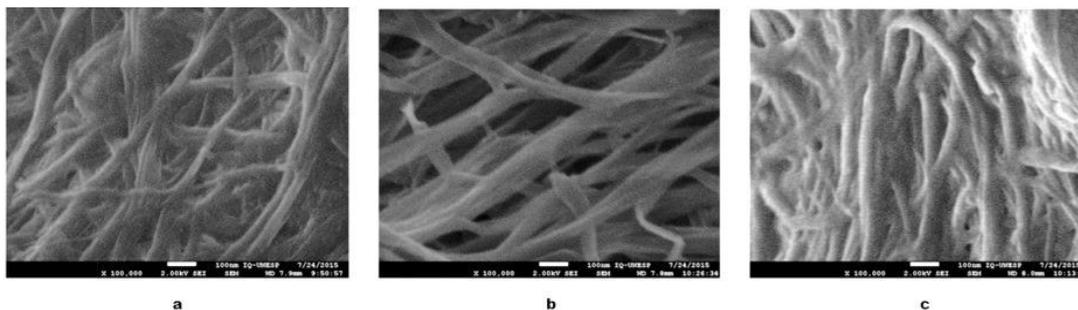
Figure 4: Scanning electron microscopy images of bacterial cellulose (a); bacterial cellulose treated with 48% sulfuric acid (b) and bacterial cellulose treated with 64% sulfuric acid (c)

Some aspects of the behavior of cellulosic fibers towards different reagents are attributed to the structure of the fiber. Cellulose is a polycrystalline aggregate containing crystalline components and amorphous components. The cellulose has a strong tendency to form intra- and intermolecular H-bonds. The existence of these links significantly influences the reactivity of cellulose, since the links of intermolecular H-

bonds increase the crystallinity of cellulose, making difficult the penetration of solvents and reagents. Conversely, more disordered (amorphous) areas facilitate the penetration of solvents and reagents, being more accessible to all chemical reactions.

The sulfuric acid solution is a good tumescence agent for cellulose, which is able to induce changes in the crystalline regions of

cellulose and increase its amorphous fraction.[26] The reduction of crystallinity can be confirmed by the results obtained from the analysis of the X-ray patterns of the samples, which is a common technique used to evaluate changes in cellulose crystallinity.[23]

The ratio of crystalline regions to amorphous ones determines the crystallinity index of the cellulose, which, in combination with the orientation of the crystalline and amorphous areas in the fiber, affects the mechanical properties of the cellulose.[24]

It is known that changes in the crystallinity of cellulose after acid treatment are directly related to the acid strengths and the hydrolysis times. The sulfuric acid is capable of breaking hydrogen bonds, with the resulting penetration into amorphous and crystalline cellulose regions.[11,24,26]

In this study, the hydrolysis time was constant and only the acid concentration was varied. The results indicate that the hydrolysis modifies the X-ray pattern by affecting the relative intensity of the peaks and the degree of crystallinity of the samples. A significant decrease in the crystallinity of cellulose with increasing sulfuric acid concentration was observed. A similar result was reported by Ioelovich[11] after the treatment of microcrystalline cellulose with 65% sulfuric acid at 45 °C (a decrease ranging between 25 and 30%). This reduction of crystallinity can be explained by extensive swelling during hydrolysis, possibly leading to disruption of the crystalline regions, with an increase of the amorphous regions, in comparison with the untreated bacterial cellulose.[20]

The slight decreases observed in the values of X (Table 3) suggest that the treated samples contain fewer cellulose chains in a highly organized form in the interior of the cellulose crystallite. The results were corroborated by FTIR spectroscopy.

This methodology allows evaluating the capacity of different absorption bands to characterize the ordering degree of cellulosic polymers. An alteration of the crystalline organization leads to a significant simplification of the spectral contour through reduction in intensity or even disappearance of the bands characteristic of the crystalline domains.[12]

During acid hydrolysis of cellulose, sulfuric acid molecules cause a breach of the H-bonds, and a partial esterification of the OH groups of cellulose occurs. Both processes contribute to the dissolution of cellulose.[11]

The hydrogen bond intensity (HBI) of cellulose is closely related to the crystal system and the degree of intermolecular regularity, that is, crystallinity, as well as the amount of bound water. In this study, a decrease in the values of HBI with increasing sulfuric acid concentration was observed. This result may be associated with a minor amount of the absorbed water in the samples treated with sulfuric acid, since the HBI value also represents the amount of the absorbed water.[29]

On the other hand, the lateral order index (LOI) and cellulose I/cellulose II ratio decrease with increasing sulfuric acid concentration. The LOI is correlated to the overall degree of order in the cellulose and it can be used to interpret qualitative changes in cellulose crystallinity, being based on the ratio of absorbance bands at specific wavenumbers. Generally, when this index decreases, crystallinity also decreases. Low LOI values indicate the cellulose treated with sulfuric acid is composed of more amorphous domains, compared with untreated cellulose. This result confirms the results obtained by X-ray analysis.

Typically, cellulose I is the most abundant phase and the most sought after due to its optimal elastic properties. The structures of conventional amorphous cellulose samples are unstable in the presence of water or moisture; they usually form partially crystalline cellulose II.[12,23] A decrease of the cellulose I/cellulose II ratio indicates that the hydrolysis process transforms the cellulose in type II.

Similar results were reported by Ioelovich,[11] who concluded that, under controlled conditions, with concentrations of sulphuric acid between 64-65 wt%, regenerated cellulose from dissolved cellulose exhibited a CII polymorph structural arrangement. The insoluble cellulose exhibits a crystalline structure CI, while the regenerated cellulose exhibits a crystalline polymorph CII.

The FTIR absorption band at 893 cm^{-1}, assigned to C–O–C stretching at β-(1,4)-glycosidic linkages, is designed an amorphous absorption band. An increase in its intensity occurs in the amorphous samples, compared to the initial ones. In this study, an increase in the intensity of this band was observed. This result indicated that the acid treatment causes an increase of the amorphous regions in the cellulose.

The evaluations by electron microscopy showed that the treatment with different concentrations of sulfuric acid modifies the structure of cellulose.

CONCLUSION

Biomaterials are an important part of the medical device industry, but their use fundamentally depends on their characteristics and structure. In this work, bacterial cellulose was modified by treatment with sulfuric acid. A decrease of the crystallinity index, facilitating solubility, was observed. The increment of the sulfuric acid concentration facilitated this process, as confirmed by XRD and FTIR analyses. A material with a different morphological surface was obtained. These changes enable the use of bacterial cellulose as biomaterial in regenerative medicine.

REFERENCES

[1] ***ANGIOSCAFF Report Summary. Angiogenesis-inducing Bioactive and Bioresponsive Scaffolds in Tissue Engineering, Lausanne, 2015, available on-line at: http://cordis.europa.eu/project/rcn/88874_en.html

[2] P. Gatenholm and D. Klemm, *MRS Bull*., **35**, 208 (2010).

[3] W. Czaja, D. Young, M. Kawecki and R. Brown, *Biomacromolecules*, **8**, 1 (2007).

[4] M. Shoda and Y. Sugano, *Biotechnol. Bioprocess Eng*., **10**, 1 (2005).

[5] P. Richmond, in "Biosynthesis and Biodegradation of Cellulose, Occurrence and Functions of Native Cellulose", edited by C. Haigler and P. Weimer, Marcel Dekker Inc., 1991, p. 23.

[6] A. Elidrissi, S. El Barkany, H. Amhamdi, A. Maaroufi and B. Hammouti, *J. Mater. Environ. Sci*., **3**, 270 (2012).

[7] G. Olyveira, M. Santos, C. Riccardi, L. Costa, P. Daltro *et al*., *Adv. Sci. Eng. Med*., **6**, 1 (2015).

[8] G. Olyveira, M. Santos, L. Costa, P. Daltro, P. Basmaji *et al*., *Sci. Adv. Mater*., **6**, 1 (2014).

[9] G. Olyveira, M. Santos, L. Costa, P. Daltro, P. Basmaji *et al*., *Sci. Adv. Mater*., **7**, 1 (2015).

[10] G. Han, S. Huan, J. Han, Z. Zhang and Q. Wu, *Materials*, **7**, 16 (2014).

[11] M. Ioelovich, *ISRN Chem. Eng*., **2012**, 1 (2012).

[12] D. Ciolacu, F. Ciolacu and V. Popa, *Cellulose Chem. Technol*., **45**, 13 (2011).

[13] M. Poletto, V. Pistor and A. Zattera, in "Cellulose – Fundamental Aspects", edited by T. Van de Ven and L. Godbout, InTech, 2013, pp. 45-68.

[14] M. Wada and T. Okano, *Cellulose*, **8**, 183 (2001).

[15] M. Nelson and R. O'Connor, *J. Appl. Polym. Sci*., **8**, 1325 (1964).

[16] M. Wada, M. Ike and K. Tokuyasu, *Polym. Degrad. Stabil*., **95**, 543 (2010).

[17] H. Barud, C. Ribeiro, J. Capela, M. Crespi, S. Ribeiro *et al*., *J. Therm. Anal. Calorim*., ESTAC2010 Special Issue, 1 (2010).

[18] L. Cabrales and N. Abidi, *J. Therm. Anal. Calorim*., **102**, 485 (2010).

[19] M. Zohuriaan and F. Shokrolahi, *Polym. Test*., **23**, 575 (2004).

[20] R. Leite de Oliveira, H. Silva Barud, R. de Assunção, C. Silva Meireles, G. Oliveira Carvalho *et al*., *J. Therm. Anal. Calorim*., **106**, 703 (2011).

[21] A. Kumar, Y. Singh Negi, V. Choudhary and N. Kant Bhardwaj, *J. Mater. Phys. Chem*., **2**, 1 (2014).

[22] E. Lengowski, G. Bolzon, S. Nisgoski and W. Esteves, *Sci. For*., **41**, 185 (2013).

[23] C. Huntley, K. Crews, M. Abdalla, A. Russell and M. Curry, *Int. J. Chem. Eng*., **2015**, 1 (2015), Article ID 658163.

[24] E. Johnson, S. Mendon, S. Thames and J. Rawlins, *J. Eng. Fiber. Fabrics*, **5**, 10 (2010).

[25] R. Swatloski, S. Spear, J. Holbrey and R. Rogers, *J. Am. Chem. Soc*., **124**, 4974 (2002).

[26] M. Ziaul Karim, Z. Zaman Chowdhury, S. Bee Abd Hamid and M. Eaqub Ali, *Materials*, **7**, 6982 (2014).

[27] M. Roman and W. Winter, *Biomacromolecules*, **5**, 1671 (2004).

[28] D. Kim, Y. Nishiyama, M. Wada and S. Kuga, *Cellulose*, **8**, 29 (2008).

[29] J. George, K. Ramana, A. Bawa and S. Siddaramaiah, *Int. J. Biol. Macromol*., **11**, 50 (2011).