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Influence of the reaction time during the treatment of bacterial cellulose with sulfuric acid solution

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ABSTRACT

Biomaterials are one of the most important parts of the medical device industry. Being used frequently in the development of Scaffolds for the tecidual regeneration. Bacterial cellulose is a biomaterial widely used in tissue regeneration. Due to its high content of hydrogen bonds, its crystallinity is high and its solubility is low, which makes its use difficult. Studies carried out with anteriority showed the modification suffered by bacterial cellulose treated with sulfuric acid solutions. The objective of this work was to study the influence of reaction time on crystallinity in the treatment of bacterial cellulose with sulfuric acid solution. Bacterial cellulose was modified by acid hydrolysis with sulfuric acid solutions of 48 or 64% for 60, 120 and 240 min. In all cases, the hydrolysed cellulose was washed with distilled water until pH 7, subsequently the cellulose was washed with ethanol and dried in an oven at 37 ° C until a constant mass. The samples obtained were characterized by X-ray diffraction and the crystallinity index, the apparent crystallite size, the crystallite inner chains and the Z-discriminant function were determined. The results showed that the reaction time has a statistically significant influence on the crystallinity of bacterial cellulose.

Keywords: *Bacterial cellulose, Crystallinity index, Acid hydrolysis, Biomaterials, X-ray.*

1. INTRODUCTION

Biomaterials are one of the important parts of the medical device industry and are now becoming more prevalent as Scaffolds in the development of sophisticated therapeutic products, such as sustained drug delivery therapy. At present, the three-dimensional (3D) printing technology (manufacture additive, rapid prototyping) as a way for the elaboration of Scaffold, is receiving significant attention in the field of Tissue Engineering and Biomaterials Science. In recent decades, there has been an increase in scientific and technological research for the development of new materials from renewable sources and potentially biodegradable [1-5].

Cellulose is the most abundant polymer on earth, its structure is predominant in plants and in some marine animals, and it can be synthesized by some fungi and bacteria. Bacterial cellulose is a polymer free of contaminants such as lignin and hemicelluloses; thereby it is an attractive biomolecule due to the ease of isolation and purification [2, 6-12].

Cellulose is a crystalline polymer and its crystallinity depends on the source and on the methods of isolation and transformation. The complex structure of cellulose is due to the hydrogen bonding, it is manifested by the existence of several polymorphs (crystalline forms) purification [6, 7]. The cellulose is renewable, biodegradable and biocompatible so it can be used as a biomaterial. Because of its high crystallinity due to the presence of large groups of hydrogen bonds, the solubility of the cellulose is low [13]. This problem can limit the use of this material.

One of the ways to eliminate this difficulty is to perform a chemical modification of the cellulose. Previous studies have shown that, through acid treatments, the cellulose crystallinity index decreased [14-17]. Recently Rodriguez-Chanfrau et al., [14], reported the treatment of bacterial cellulose with sulfuric acid solution at concentrations of 48 and 64% for one hour, showing that the solubility of the cellulose was increased by approximately 18% compared with the untreated cellulose.

The objective of this work was to evaluate the influence of reaction time on the crystallinity index of bacterial cellulose treated with sulfuric acid solution.

2. MATERIALS AND METHODS

Bacterial cellulose (Innovatecs Products Biotechnological LTDA, São Carlos - São Paulo, Brazil) was modified by the method described by Rodriguez-Chanfrau et al., [8]. Sulfuric acid solutions (Merck, Germany) were used at 48% or 64%. The acid treatment was carried out at room temperature (32 ± 2 ° C) with constant agitation. Times of 60, 120 and 240 min were studied. In all cases, at the end of the acid hydrolysis process, the cellulose was washed with distilled water until pH 7. Subsequently the cellulose was washed with ethanol and dried in an oven at 37 ° C until a constant mass. The experiment was carried out in triplicate. The determination of yield and solubility were carried out according to Rodriguez-Chanfrau et al., [14].

X-ray powder diffraction studies.

The 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), Flicker detector and graphite monochromator. The scattering angle range from 4º to 80º with 2θ step interval of 0.02º was used.

Cellulose samples were cut into small pieces and laid on the glass sample holder, analyzed under plateau conditions. An operating voltage of 40 kV and current of 30 mA was utilized, and the intensities were measured in the range of $5° < 2\theta < 30°$. Peak separations were carried out using Gaussian deconvolution. The d -spacings were calculated using the Bragg equation. The analysis was performed in triplicate.

The crystallinity index (IC) of the treated cellulose samples was estimated according to Ciolacu et al., [18]. The apparent crystallite size (L) was determined using the Scherrer equation [19, 20].

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 the following equation: $X = (L - 2h)^2/L^2$: where L is the apparent crystalline size for the reflection of plane (200) and h is the layer thickness of the surface chain (0.57 nm) [21].

The Z-discriminant function was estimated according to Wada and Okano, [22]; where if $Z > 0$ indicates Iα; while $Z < 0$ indicates the Iβ dominant type.

Statistical analysis.

To determine the influence of reaction time (factor) on crystallinity index of the treated bacterial cellulose with sulfuric acid solution (response variable), a statistical analysis using one-way ANOVA was performed. All the data using Statgraphic plus 5.1 (USA) statistical software were processed. The results were considered significant when $p < 0.05$.

3. RESULTS

Table 1 presents the results of the determination of yield and solubility. A yield higher than 80% was observed for each of the treatments applied.

Table 1. Results of the analysis of yield and solubility for samples treated at different time.

Treatments (%)	Time (min)	Yield (%)	Solubility (%)
48	60	81,8	18,2
	120	80,9	20,1
	240	81,9	18,1
64	60	81,9	18,2
	120	80,9	19,1
	240	80,8	18,9

This result was considered adequate for the scale of work studied. On the other hand, the solubility values were between 18 and 20%. These results indicate that the reaction time does not influence the aforementioned variables. Similar results were obtained previously [14].

Figure 1 shows the X-ray diffractogram of untreated bacterial cellulose and bacterial cellulose treated with 48% sulfuric acid solution at the time studied. Peaks of diffraction are observed at $2\theta = 14.0°$; 16.2°; 22.5° and 35.3°, typical of cellulose (PDF 502241). It is also observed that, as the reaction time increases, the intensity of the peaks decreases.

Figure 2 shows the X-ray diffractogram of bacterial cellulose treated with 64% sulfuric acid solution. Similar to the difractograma previously shown, the characteristic peaks of the cellulose was observed, observing that the intensity decreases as the reaction time increases. In this case, the decrease in intensity is greater than the decrease in the intensity of the peaks in the samples treated with 48% sulfuric acid solution. Similar results were observed in previously published studies [14].

Table 2 shows the results of the determination of apparent crystallite size (L), the proportion of the crystallite inner chains (X) and Z-values.

The results of the determination of apparent crystallite size (L) showed a decrease when the cellulose was treated with 48% and 64% sulfuric acid solution. No appreciable difference was observed between the values of the samples treated at different times with 48% sulfuric acid solution and the values obtained in the samples treated at different times with 64% sulfuric acid solution.

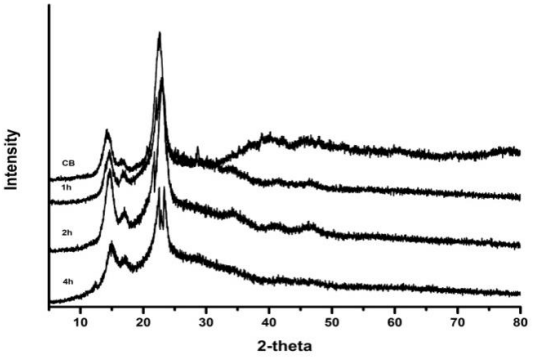

Figure 1. X-ray diffractogram of bacterial cellulose treated with 48% sulfuric acid solution.

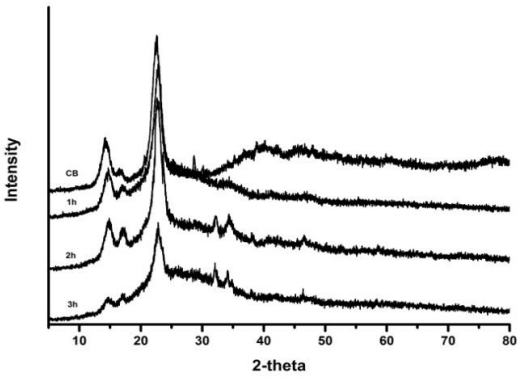

Figure 2. X-ray diffractogram of bacterial cellulose treated with 64% sulfuric acid solution.

On the other hand, the results of the determination of the proportion of the internal chains of crystallite (X) showed in all cases a slight decrease with respect to untreated cellulose. Whereas, the determination of the Z value indicates that the

treatment transforms the cellulose of a dominant type Iα (Z> 0) into a dominant type Iβ (Z <0).

Table 2. Parameters obtained from the XRD analysis of the samples studied.

Treatments	Time (min)	L 200 (nm)	X	Z
untreated		2.98	0.3954	20.5
48%	60	1.42	0.3113	- 23.7
	120	1.47	0.3095	- 23.3
	240	1.47	0.3054	- 23.9
64%	60	1.46	0.2577	- 26.0
	120	1.48	0.2510	- 25.9
	240	1.57	0.2409	- 26.3

Figure 3 shows the behavior of the crystallinity index over time. The increase in the reaction time decreases the crystallinity of the cellulose. This decrease is greater in samples treated with 64% sulfuric acid solution. Statistical analysis showed that the decrease in crystallinity was statistically significant in both treatment groups ($p = 0.023$ and $p = 0.015$ for samples treated at 48% and samples treated at 64% sulfuric acid solution, respectively). The comparative analysis between the treatment groups showed that there were statistically significant differences between them ($p = 0.004$).

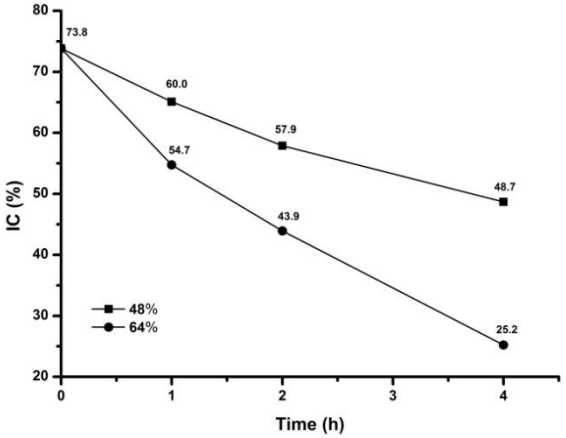

Figure 3. Influence of the reaction time on the crystallinity index.

An analysis of the results showed that treatment with sulfuric acid solution at 48% causes a decrease in the crystallinity index of the treated cellulose above 18% (Table 3). Whereas, as the concentration of the acid solution increases to 64%, the decrease in the crystallinity index increases.

Table 3. Percentage of decrease in the crystallinity index over time.

Treatments	Time (min)	% decrease
48%	60	18.7
	120	21.6
	240	34.0
64%	60	25.9
	120	40.5
	240	65.9

Cellulose is composed of crystalline parts and amorphous parts, in proportions that vary according to their source of production, with the majority being the crystalline portion in most cases. The proportion that exists between the amorphous region and the crystalline region define the physical and chemical properties of the material. Being in the amorphous region reactions of this material usually occur. Sulfuric acid is a dehydrating agent, which, through an esterification process, removes the OH groups from the cellulose, decreasing the crystalline zones and increasing the amorphous zones within the material [23-26].

The degradation of cellulose begins with the formation of swelling on its surface. The diffusion of water favors the reduction of the particles in the polyglucose chains by partially converting the crystalline parts into amorphous parts [26]. Therefore, it is expected that the contact time between this solution and the cellulose will favor the penetration of the solution into the polymer structure, affecting the crystallinity of the material.

The results of this study corroborate that the reaction time during the process of cellulose modification with sulfuric acid solution, significantly affects the crystallinity index of the material studied. Modifying also, other material parameters such as the crystalline size and the Z value. It was further confirmed that the increase in the concentration of the sulfuric acid solution significantly favors the decrease in the crystallinity index of the sample.

4. CONCLUSIONS

One of the ways to improve the solubility of cellulose is its chemical modification by acid treatment. In this study, the influence of the reaction time on the bacterial cellulose crystallinity index was evaluated. It was concluded that the reaction time significantly influences the crystallinity index of cellulose treated with sulfuric acid solution in concentrations of 48 and 64%. It was not observing the same behavior for the performance and the solubility.

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