Microfibrillated Cellulose from Sugarcane Bagasse as a Biorefinery Product for Ethanol Production

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Received November 02, 2017; Accepted January 31, 2018

ABSTRACT: Research involving the preparation of microfibrillated cellulose (MFC) from sugarcane bagasse is a relevant topic to the production of new nanomaterials and more accessible cellulose substrates for the production of second generation ethanol. Regarding the transformation of cellulose into glucose, the precursor of second generation ethanol, this nanosized cellulosic substrate represents a more appropriate material for the chemical hydrolysis process. The high aspect ratio of MFC improves hydrolysis, requiring mild conditions and decreasing the generation of by-products. Here, MFC was prepared from sugarcane bagasse by ultrasound defibrillation. This material was oxidized with 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) to produce negatively charged high defibrillated MFC. The MFC was characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and viscosity measurements.

KEYWORDS: Cellulose nanofibrils, substrate, second generation ethanol

1 INTRODUCTION

Sugarcane represents a major raw material for the agricultural industry in Brazil, producing sugar, ethanol, fuel and energy from bagasse. Energy generated from bagasse is used in the ethanol plant and the excess is transformed into electricity. Ethanol is produced by a biotechnological route using the sugar juice as a source of carbohydrates for fermentation. The largest volumes of ethanol are used as biofuel, used in the hydrated form in ethanol engines or in a mixture (about 20–25%) with gasoline [1–3]. The sugarcane industry is considered one of the largest biorefinery operations in the world. The use of sugarcane bagasse for other applications besides fuel to generate energy has grown, and more recently has attracted great attention due to the large volumes available, both for the production of new materials and for the production of second generation ethanol [4, 5]. For ethanol production by fermentation, the cellulose should be converted into glucose, the source of energy for microorganisms.

Despite the fact that most of the investment in second generation ethanol is focused on the enzymatic process, an alternative promising way is the chemical process based on acid hydrolysis [5]. Acid hydrolysis can be faster than enzymatic hydrolysis and very efficient if cellulose is easily accessible; however, nonhomogeneous raw materials, such as the cellulosic substrates from sugarcane bagasse, can lead to low yield and the formation of by-products.

Here we propose the pretreatment of sugarcane bagasse to obtain fine and homogeneous material, MFC (or nanocellulose), a natural polymer with a promising future [6], as the substrate for second generation ethanol. The acid hydrolysis of MFC tends to be fast and complete, leading to higher yields when compared to the untreated bagasse [7–9], once the access of chemicals to the nanofibers is improved, because of its nano- and micrometric dimensions.

Certainly, the production of ethanol from MFC is not economically viable as a substrate for ethanol production; however, this study can lead to the development of new and more rational pretreatment methods of lignocellulosic materials prior to acid hydrolysis in the future. In this vein, this work shows the production of cellulose nanofibers (MFC) from sugarcane bagasse and its characterization. The MFC were produced by

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DOI: 10.7569/JRM.2018.634109

bleaching and treating the sugarcane bagasse with a high-power ultrasound. They were further oxidized with 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO reagent) to produce charged MFC. The MFC were characterized by SEM, FTIR, XRD and their hydrogels were characterized by rheology. The purpose is to evaluate the use of bagasse for both the production of an advanced material (MFC) and in the future its use as a model material to study acid hydrolysis of cellulose.

2 EXPERIMENTAL

2.1 Materials

2,2,6,6-Tetramethyl-1-piperidinyloxy radical (TEMPO) was purchased from Sigma-Aldrich; sodium bromide, sodium chloride, potassium hydroxide and sodium hypochlorite were purchased from Synth. Sugarcane bagasse was provided by Usina São José S/A Açúcar e Álcool, Rio das Pedras, São Paulo, Brazil.

2.2 Characterization of the Sugarcane Bagasse

Sugarcane Bagasse Sampling: A representative fraction of fibers of sugarcane bagasse was washed in water and dried at room temperature until 10% moisture. The chemical composition of the resulting bagasse was determined (three replicates), and then it was milled using a universal cutting mill (Pulverisette 19 and Pulverisette 14, Fritsch), until a granulometry range of 0.5–0.2 mm [10].

The moisture was determined using a Sartorius model MA35 heating balance. The ash content was determined gravimetrically by burning the sample and weighing the residue according to NREL procedure, "Determination of Ash in Biomass" [11]. The extractives content was determined by Soxhlet extraction in accordance with TAPPI T 204 cm-97 [12].

Lignin and carbohydrates content were determined according to the protocol, "Determination of Structural Carbohydrates and Lignin in Biomass" [13]. The insoluble portion of the extraction was submitted to a two-stage acid hydrolysis consisting of a first stage 72 wt% sulfuric-acid treatment in a 30 °C water bath for 1 h with frequent stirring, followed by a second stage applying 4 wt% sulfuric acid in an autoclave for 1 h at 121 °C. After cooling, sample was filtered in a sintered glass funnel, previously weighed. The funnel, containing solid fraction, was heated at 105 °C until constant weight. Final dried mass, subtracted out of its ash and extractives contents, is related to insoluble lignin. The filtrate was characterized by liquid chromatography (HPLC) to determine concentrations of cellobiose, glucose, xylose, arabinose, galactose, hydroxymethyl furfural, furfural and acetic acid. With corrections for stoichiometry and dilution, concentrations of glucose, cellobiose, and hydroxymethyl furfural were converted to cellulose content in the solid. Similarly, concentrations of xylose, arabinose, furfural and acetic acid were converted to hemicelluloses' content. Soluble lignin in the filtrate was determined from ultraviolet absorption (280 nm) according to Gouveia *et al.* [14].

2.2.1 *MFC from Sugarcane Bagasse*

The process for the preparation of microfibrillated cellulose was based on four steps: i) pre-extraction with toluene/ethanol solution, ii) bleaching with sodium chlorite solution, iii) extraction of hemicelluloses with hot KOH solution and iv) sonication with a high-power ultrasound [7, 15]. Before pre-extraction, sugarcane bagasse was washed with hot water, dried and milled to 0.25 mm and 0.04 mm sizes using an IKA MF-10 mill with stainless steel sieves.

Pre-extraction: The extracting was performed with toluene/ethanol 2:1 (v/v) for 8 h. The extracted bagasse was dried at room temperature for 4 h and then in the oven at 75 °C for 1 h. The dried material was weighed (weight loss of ~3%).

The fibers were dispersed in 200 mL of 1,3% sodium chlorite solution, pH ~4. The mixture was poured into an Erlenmeyer flask attached to a reflux condenser and kept at 75 °C under magnetic stirring for 1 h. After this period, the suspension was filtered under reduced pressure and the filtered fiber replaced in the Erlenmeyer flask with a further 200 mL of fresh sodium chlorite solution and the procedure was repeated once. The final fiber was filtered and washed with distilled water.

The treated fibers were then added to a KOH solution prepared using 5 g of KOH and 250 mL of distilled water. The solution was stirred at 85 °C for 2 h. Then, the fiber was filtered and washed with a large volume of distilled water up to neutral pH. This procedure was repeated twice. After that, the fibers were dispersed in 200 mL of distilled water and cooled in an ice bath.

The sonication was held using a Hielscher sonicator apparatus UP400S (22 kHz and 400 W) equipped with a cylindrical titanium alloy probe of 40 mm diameter. The optimized condition for sonication was the total period of time of 40 min, for which the equipment was turned on for 5 min, followed by 5 min of resting without sonication. The pulses were applied for 10 s followed by 10 s resting without pulses. The sonication was carried out using an ice bath to avoid the sample heating.

2.2.2 *Oxidation of MFC from Sugarcane Bagasse*

TEMPO-oxidized cellulose nanofibrils (To-MFC) were prepared following the method described by Saito *et al.* [16]. Briefly, the nanofibers obtained in the third step (iii) of the previous method, i.e., before sonication, were dispersed in water at 2 wt%. Sodium bromide (1 mmol/g cellulose) and TEMPO reagent (0.1 mmol/g) were added, followed by the slow addition of sodium hypochlorite (NaClO) (7 mmol/g cellulose), and the pH of the suspension was adjusted to 10 by adding 0.1 M NaOH. The pulp was washed with deionized water to neutral pH. The oxidized fibers were sonicated in an ultrasonicator (22 kHz, 400 W, equipped with a cylindrical titanium alloy probe of 40 mm diameter) until the formation of a transparent gel. The obtained gel was then filtered by passive ultrafiltration (dialysis).

2.3 Characterization

The MFC content was determined by gravimetric analysis at 105 °C for 4 hours, the first hour being with forced air circulation.

Infrared spectroscopy was performed on dried and hot-pressed samples obtained after sonication using a PerkinElmer Spectrum 100 spectrometer in ATR mode. Spectra were acquired after 64 scans in the range of 650 to 4000 cm^{-1}.

X-ray diffraction: MFC were analyzed on a Carl Zeiss Jena URD-6 X-ray spectrometer using Cu Kα radiation in the range of 4° 2θ to 40 2° θ in steps of 1.2°/min. The samples were prepared by casting the MFC suspension on a glass plate and the dried film was used for the analysis. X-ray diffractogram was used to calculate the crystalline index of MFC, To-MFC and the sugarcane bagasse using the Segal method [17], which is based on subtracting the amorphous band *I_am* (minimum intensity of the peak 101) from the crystalline portions (*Ic*) of the peak 002 (maximum intensity) and then taking the ratio between the difference and total intensity [2], following the equation:

$$CI = (Ic - I\_am)/Ic*100$$

where CI = crystallinity index, Ic = maximum intensity, I_am = minimum intensity of the peak 101.

Scanning electron microscopy (SEM) was performed in an FEI Inspect F50. The MFC suspension was deposited on the surface of an aluminium polished support, dried and coated with evaporated platinum; To-MFC samples were freeze-dried, glued at the surface of a carbon conductive tape and coated with evaporated platinum. The TGA thermograms were obtained using a PerkinElmer Pyris 1 instrument. Each sample (10–20 mg) was placed in a platinum crucible and heated from 25 °C to 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

Viscosity: The assays were carried out using a TA Instruments AR 1000N controlled strain oscillatory rheometer, equipped with a cone-plate (20 mm and 2° and 0.69 mm gap) geometry. The shear rate ranged from 0,1 to 1000 1/s, at 25 °C. The solid content of MFC and To-MFC aqueous suspensions was 0.5 %wt.

3 RESULTS AND DISCUSSIONS

3.1 Chemical Composition of Depithed Sugarcane Bagasse

The chemical composition of depithed sugarcane bagasse is showed in Table 1. The results are in agreement with the composition of sugarcane bagasse found in the literature [18–22].

The general aspect of the cellulose-based hydrogels is showed in Figure 1. Figure 1a–d shows a 0.5 wt% MFC aqueous suspension which is a gel-like material. The material is translucent, and no pulp agglomeration was observed, as one can see in Figure 1d. Figure 1e,f shows the transparent, high viscous and homogeneous aspect of TEMPO-modified MFC (To-MFC) suspension at 0.5 wt%.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 2 shows the FTIR spectrum of the dried MFC and To-MFC samples, which correspond to cellulose, as expected, once the method generates pure and ultrafine cellulose nanofibers. The spectra showed strong bands at around 3300, 2880 and 1100 cm^{-1}, corresponding to the vibrations of the O-H, C-H and C-O groups of cellulose, respectively. The To-MFC spectrum showed, in addition to the typical peaks of cellulose, a strong peak at 1600 cm^{-1} corresponding to COOH group [23], indicating the success of the oxidation method.

Table 1 Composition of sugarcane bagasse.

Components	**Content (wt%)**
hemicelluloses	29.87 (± 2.46)
cellulose	43.17 (± 3.67)
total lignin	22.57 (± 3.13)
extractives	3.33 (± 0.01)
ash	1.61 (± 0.24)

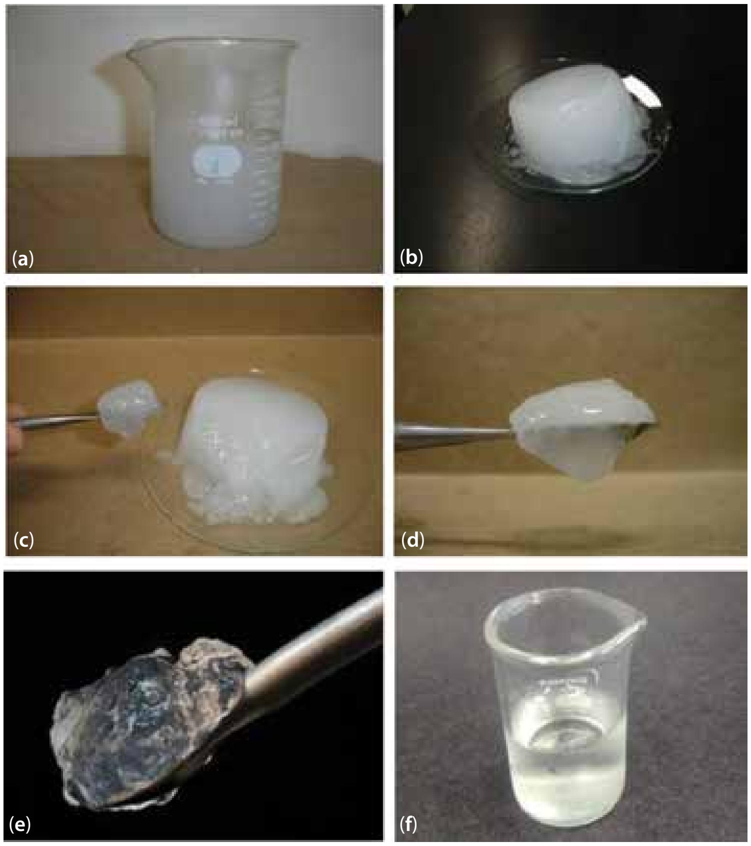

Figure 1 MFC aqueous suspension at 0.5 wt% of (**a**–**d**) non-oxidized MFC and (**e**,**f**) TEMPO-oxidized MFC prepared from the previous material (**a**–**d**).

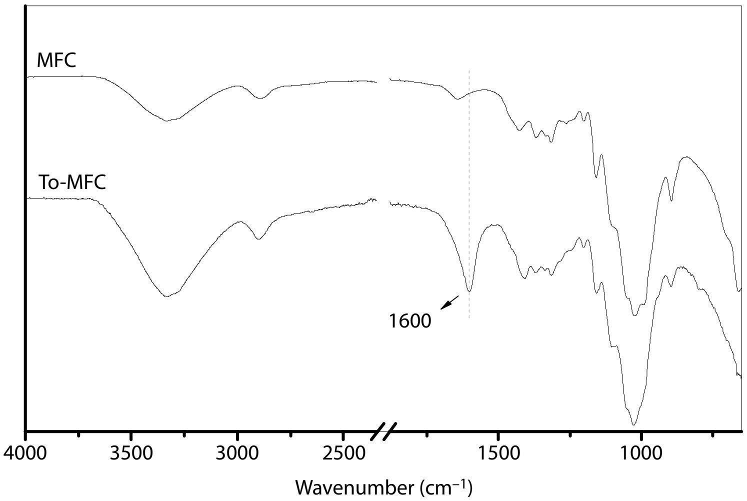

Figure 2 FTIR spectra of MFC and To-MFC.

3.3 X-ray Analysis

Figure 3 shows the X-ray diffraction (XRD) patterns of sugarcane bagasse, cellulose and MFC. As can be seen from Figure 3, all the samples showed three reflections characteristic for cellulose at 2θ ~16°, 23° and 34.5°, corresponding to the (1 1 0), (2 0 0), and (0 0 4) crystallographic planes of cellulose I [24]. These results show that crystal structures of the cellulosic substrates remained unchanged during TEMPO oxidation. The

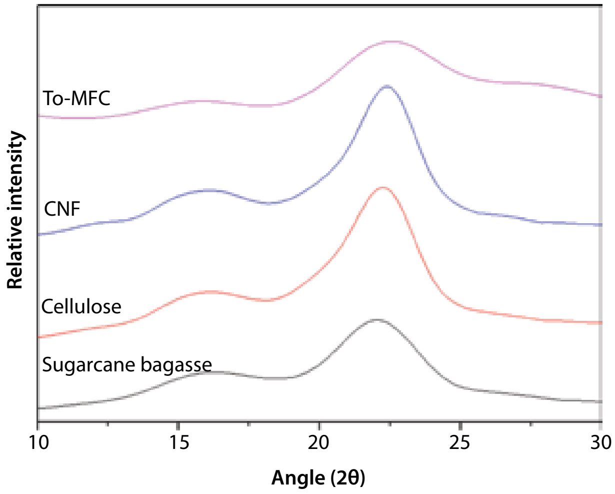

Figure 3 XRD patterns of sugarcane bagasse: Cellulose, CNF and To-MFC.

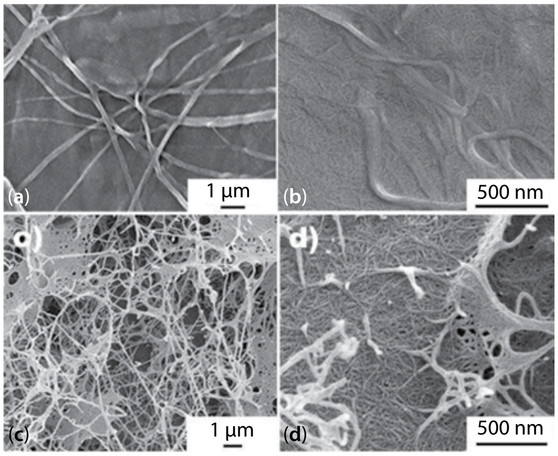

Figure 4 SEM micrographs of the surface of dried MFC film at two magnifications with scale bar of 1 µm (**a**) and 100 nm (**b**); and To-MFC (**c** and **d**) with scale bar of 1 µm and 500 nm.

estimated crystallinity index was 47% for To-CNF, 71% for CNF, 73% and 57% for sugarcane bagasse. These data are in agreement with the literature [25, 26]. The higher crystallinity of Cellulose and MFC and To-MFC fibers is probably ascribed to the removal of noncrystalline disordered regions during the microfibrillation process.

3.4 Scanning Electron Microscopy (SEM)

Figure 4 shows the SEM images of the surface of cast films of MFC and To-MFC. A few larger fibers with ~200 nm diameter are visible in Figure 4a, the majority of the fibers with reduced diameter being less than 20 nm (Figure 4b). To-MFC also showed small diameter fibers (about 20 nm) and high homogeneous aspect, as can be seen in Figure 4c. These images showed that, in fact, the microfibers produced from sugarcane bagasse are very thin and homogeneous material, offering a very interesting material for chemical modification and highly efficient acid hydrolysis.

The results of SEM (Figure 4) shows the homogeneous aspect of cellulose nanofibers separated from cellulose fibers, with average diameter of about 20 nm. The amount of cellulose macromolecules exposed at the surface of the low diameter nanofibers is high, providing a homogeneous substrate for the chemical hydrolysis, which can be seen as a promising alternative route in order to overcome the drawbacks of this process. The idealized molecular arrangement of the cellulose chains at the surface of the cellulose nanofiber is schematically shown in Figure 5, which gathers information to help readers better understand the improved accessibility provided by the nanofibers to the chemical hydrolysis.

3.5 Rheology of MFC Suspensions

The viscosity as a function of shear stress of both MFC and To-MFC nanocellulose samples were quite similar, showing decreased viscosity with increasing the shear force, as shown in Figure 6. The shear thinning behavior is typical of nanocelluloses and can be explained by the chemical structure of the cellulose macromolecule, which is in agreement with the literature [27, 28]. The shear thinning behavior is typical of nanocelluloses and can be explained by the chemical structure of the cellulose macromolecule. Cellulose is rich in OH (or COOH groups in the case of To-MFC) capable of keeping the high viscous gel-like structure by the high number of hydrogen bonds; however, these hydrogen bonds break when shear force is applied, leading to the decrease of viscosity. The high viscosity is the result of the homogeneous and low-dimensional nanofiber suspensions.

3.6 Thermal Analysis

Thermogravimetric analysis of the cellulosic substrates was carried out to assess their thermal stability and degradation profiles. The TGA tracing of MFC displayed a single weight loss step, with maximum decomposition rates at around 320 °C (Figure 7). This behavior reflected the typical unzipping process of polymers being heated well above their monomer ceiling temperature. To-MFC displayed two weight loss steps with a maximum decomposition at 260 °C (about 75%) and a further weight loss at 295 °C (about 25%), which can be attributed to the modified

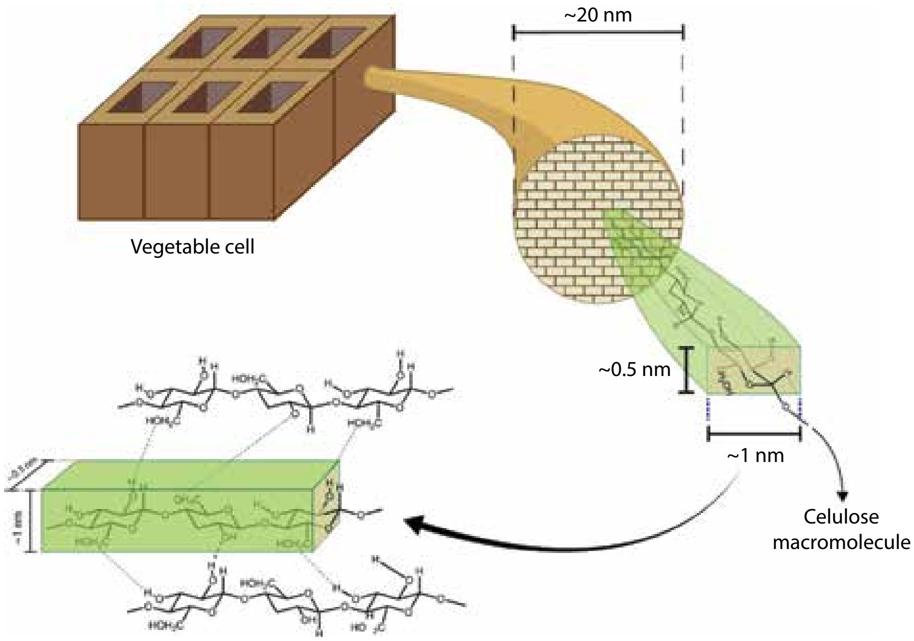

Figure 5 Schematic representation of a cellulose fiber and the nanocellulose microfibrils with 20 nm diameter. Each cellulose chain forms a nearly flat tape structure with a cross-section area with a dimension of 1×0.50 nm^2.

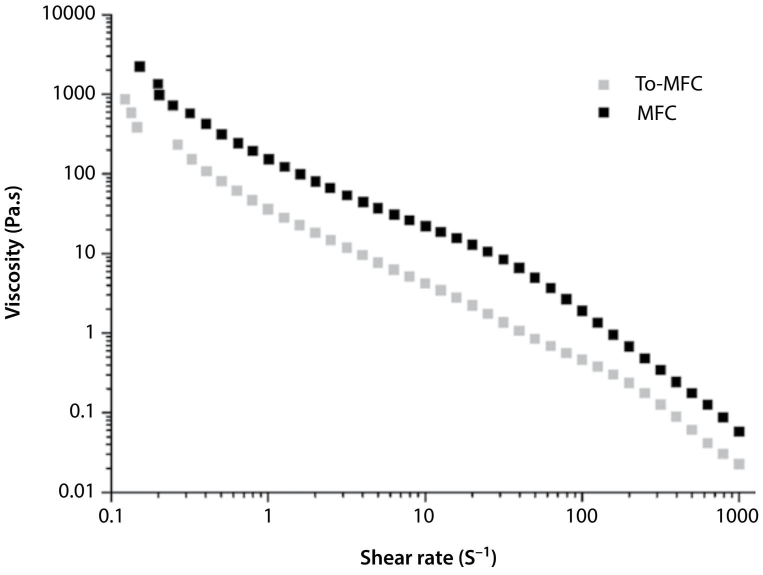

Figure 6 Viscosity as function of shear rate for nanocellulose suspensions.

fractions of cellulose and to the unmodified fractions, respectively.

4 CONCLUSIONS

Microfibrillated cellulose (MFC) gels from sugarcane bagasse were prepared by high-power ultrasound sonication. Even at low concentrations, such as 0.5 wt%, the gels showed a high consistency, evidencing the formation of a network. Visual observation corroborated by scanning electron microscopy analysis showed that only nanofibers are present. The morphology of the nanocelluloses observed by SEM indicated a network of thin nanofibers. TEMPO-modified

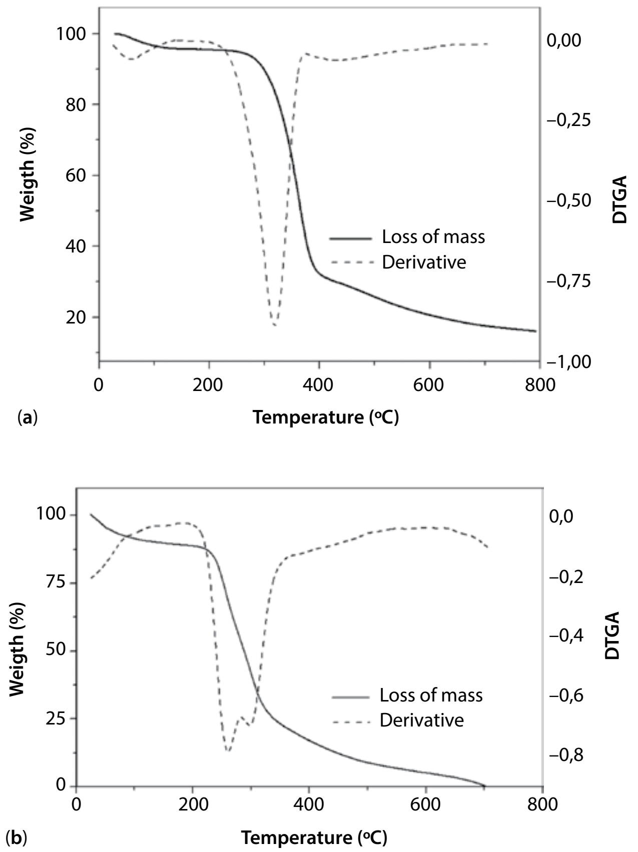

Figure 7 Thermal analysis of MFC (**a**) and To-MFC (**b**).

MFC were prepared and characterized. Rheology results showed that MFC and To-MFC showed similar rheological behavior. Nanocelluloses are being used in several applications and low cost and low energy-consuming processes are of great interest for the use of cellulose as raw material for chemical modification, including its conversion by acid and enzymatic hydrolysis in ethanol and other biorefinery products.

ACKNOWLEDGMENTS

The authors acknowledge CNPq for financial support, FAPESP for a postdoctoral fellowships of R. G. (2015/07744-0) and Marília Marta Horn for viscosity measurements.

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