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Isolation and Characterization of Cellulose Nanofibers from Argentine Tacuara Cane (*Guadua Angustifolia Kunth*)

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Abstract: New trends in the area of material improvement are the use of natural nano-charges from renewable biomass, improving the value and sustainability of our country's natural products. Bamboo is widely used in many countries of the world, although in Argentina, despite being commercialized and exported for the manufacture of wood floors, it goes unnoticed despite having native species. Therefore, researchers identified the native and exotic species present in our country and are working on novel uses. In this context, it is proposed the Argentine Tacuara Cane (*Guadua Angustifolia Kunth*), endemic plant as a new source of nanocellulosic materials, where stem fibers have been isolated using a green method achieving with yield of 45.9% of cellulose. The cellulose nanofibrils (CNF) were obtained using a green homogenization method. The CNF exhibited web-like long fibrous structure with the diameter of 10-20 nm. The crystallinity was 65.5%, as for the onset temperature of thermal decomposition was 212°C. The nanocellulose isolated from the Tacuara Cane seed fibers has a high potential to be used as a new source of cellulose-based nanofiller for the reinforcement of bionanocomposite films.

Keywords: Cellulose; nanofibers; green valorization

1 Introduction

The field of biopolymers has gained great interest in the world in the last decades, to replace the oil products due to high cost and to produce eco-friendly materials [1]. This is why the concept of "green composites" is a solution that combines polymers called "bio-based" with renewable reinforcements, resulting in sustainable materials [2]. The use of biomass fibers to reinforce plastics has several advantages over synthetic ones. They offer environmental benefits because of their renewable nature and low energy consumption in production. They are low cost, low density, have a high specific strength and modulus, and are comparatively easy to process due to their nonabrasive nature [3].

Cane Tacuara Guadua is the most important native bamboo genus in tropical America; it is endemic to this continent, with approximately 30 species distributed from Mexico to Argentina, which can be found in an altitude range that goes from sea level to 2,200 m [4].

The exploitation of this species has intensified markedly. It is used for the elaboration of cellulose, reaching great economic importance [4,5].

Cellulose is a renewable, biodegradable and non-toxic material. It is also a great source of environmentally friendly and biocompatible products, and it is considered to be the most abundant renewable polymer on Earth. The biopolymer consists of a linear polysaccharide composed of β-D-

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glucopyranose units linked by β-1-4 bonds [6,7]. The hydroxyl groups present in its structure are capable of forming hydrogen bonds, which play an important role in the direction of crystalline packing and also govern the physical properties of cellulose. Cellulose fibers exhibit a unique structure hierarchy derived from their biological origin. They consist of sets of nanofibers with a diameter of 2-20 nm and a length of more than a few micrometers [8-10].

Cellulose nanofibers (CNF) as a new cellulosic material was introduced by the research group of Turbak in 1983, and research group of Herrick in same year who produced cellulose with lateral dimensions in nanometer range by passing a softwood pulp aqueous suspension several times through a high-pressure homogenizer [11,12]. During such treatment, strongly entangled networks of nanofibrils, having both crystalline and amorphous domains, are produced due to high shearing forces. They possess high aspect ratio and form gels in water with shear-thinning and thixotropic behavior. Suspensions of CNF are biodegradable and have no cytotoxic effects. Incorporation of CNF in materials can increase tensile strength and thermal stability [13]. Taking into account the above, the objective of the work was the isolation and characterization of cellulose nanofibers from Tacuara Cane.

2 Experimental

2.1 Materials

The Tacuara Cane (*Guadua Angustifolia Kunth*), of 2-3 years old, was obtained from the banks of the Tigre City delta located in Buenos Aires, Argentine. The stems were cut in small pieces of approximately 3 × 3 cm; these pieces were processed using a blender. The chemical products (toluene, ethanol, NaOH and H2O2) used to extract the cellulose from the cane was of analytical quality and was obtained from Sigma-Aldrich. Deionized water was used in all experiments.

2.2 Isolation of Cellulose and Nanofibers Cellulose (CNF) from Tacuara Cane

2.2.1 Isolation of Cellulose

The pulp was repeatedly washed with water and dried at controlled temperature (45°C for 24 h). The pulp (100 g) was firstly treated by a mixed solution of 500 mL ethanol:toluene solution (2:1) in a Soxhlet extractor at 90°C for 6 h, with the object to remove some compounds, such as chlorophyll, wax, fatty acid, tannin, and pigment. After this process, the treated pulp was removed from the Soxhlet extractor and dried at 45°C for 3 h [14-16]. Then, the waste was treated with 500 mL of NaOH solution (2%) at 75°C for 30 min to partially remove hemicellulose and lignin. Thereafter, the residual was filtered and rinsed with deionized water four times until neutral pH. A subsequent bleaching was carried with 500 mL of hydrogen peroxide solution (10%) at 60°C for 6 h. The fibers were subsequently dried for 24 h at 40°C in a convection oven [17].

2.2.2 Preparation of Cellulose Nanofibers (CNF)

The pulp was first disintegrated by an IKA high-shear mixer (T-T18 ULTRA TURRAX Basic) at 15000 rpm speed using pulp suspensions of 2% consistency. The fibers were then refined using ultrafine friction grinder or a so-called supermasscolloider (MKCA6-2, Masuko Sanguo, Japan) and were passed through the device up to 60 times. The gap between the disks was adjusted to 9 μm. CNF was centrifuged at 10,000 rpm to reduce its water content and kept wet in the fridge [13,18].

2.3 Characterization

2.3.1 Chemical Characterization of Cellulose

To determine the chemical composition of cellulose Tacuara Cane (after chemical treatment), the Technical Association of Pulp and Paper Industry (TAPPI) standards TAPPI T222 om-02 for lignin and

TAPPI T203 cm-99 for hemicelluloses and cellulose were used. The detailed procedures are discussed in later sections.

2.3.2 Field Emission Scanning Electron Microscopy (FESEM)

The surface morphology of the fibers and nanoparticles samples were observed using field emission scanning electron microscopy (FESEM) using a Zeiss DSM982 Gemini. Test samples were coated with gold using a vacuum sputter coater before subjected to the FESEM analysis.

2.3.3 Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

Raw materials and purified cellulose were analyzed using attenuated total reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy (Nicolet IS50) to examine the changes in functional groups induced by purification of cellulose and to compare them among samples. Spectra were collected in the range of 4000 cm^{-1}-500 cm^{-1}, at a resolution of 4 cm^{-1}.

2.3.4 X-Ray Diffraction Analysis

A Siemens D 5000 X-ray diffractometer was used to observe the diffraction patterns of all the developed films. X-ray generator tension and current were 40 kV and 30 mA, respectively. The radiation was Cu *K*α of wavelength 1.54 Å. The diffraction patterns were obtained at room temperature in the range of 2θ between 10° and 40° by step of 0.02°. The crystallinity index (CrI) of cellulose was calculated from XRD pattern according to the Segal method [19]:

$$CrI(\%) = \frac{I_{002} - I_{am}}{I_{002}} x100$$

where I_{002} is the maximum intensity of the (002) lattice diffraction at 2θ = 23.0° and I_{am} is the intensity of diffraction at 2θ = 18° [19].

2.3.5 Thermal Characterization

A simultaneous thermogravimetric/differential thermal analyzer (TGA/DTA DTG-60 Shimadzu instrument, Kyoto, Japan) was used to evaluate the thermal properties of cellulose and CNF. Approximately 5 mg of each sample was subjected to heating from 30°C to 500°C a rate of 10 °C min^{-1}. Thermal properties were evaluated from the curves of both TGA and DTA.

2.3.6 Differential Scanning Calorimetry (DSC)

Calorimetric measurements were made on a TA Q20 differential scanning calorimeter in a dry nitrogen atmosphere. Indium standard was used for calibration. Samples of 5-10 mg were placed in the DSC pan. Samples were first heated to 200°C and held at that temperature for 10 min to remove the thermal history. Then, samples were cooled to -25°C at a rate of 10 °C/min, held for 10 min, and again heated to 200°C at 10 °C/min. The Tg values were taken as the midpoint of the transition in the second heating scan.

3 Results and Discussion

3.1 Isolation and Characterization of Cellulose

3.1.1 Purification and Chemical Composition of Cellulose

The isolation process starts with the extraction with different solvents to eliminate the chlorophyll, wax, fatty acid, tannin, and pigments [14]. Then, it was proceeded to add a diluted alkali with the objective to remove the lignin, hemicelluloses, waxes, pectin, proteins, soluble mineral salts, silica and ash, while that the peroxide bleaching treatment was applied to remove the lignin residues [20]. The yield

of purification was 45.9%, the mass loss of cellulose during the purification process can be attributed to two factors: (i) the breakdown of cellulose chains during bleaching treatment and (ii) the loss of material inherent in various filtering processes and mass transfer [21]. The pulp obtained after the chemical treatments, we obtained cellulose: hemicelluloses: lignin in a relationship in percentage 87.6: 9.8:1.6, respectively (the values are based on dry basis).

Fig. 1 shows the physical aspect of the Tacuara Cane during different purification steps. Visually, variations in pulp coloration are observed when subjected to different treatments, resulting in a white pulp.

Figure 1: Photographs of **A:** the Tacuara Cane, **B:** pieces of cane cut untreated, **C:** delignified cane pulp, and **D**: bleached pulps

3.1.2 FESEM Observations of Cellulose

Scanning electron microscopy images of the Tacuara cane fibers at different processing stages are shown in Fig. 2. In micrograph **A** is observed the smooth surface of raw fiber showing large fiber bundles and intact structures. The fibers are composed by several microfibrils with diameters in the range between 5-10 μm. Each elementary fiber shows a compact structure; exhibiting an alignment in the fiber axis direction, with some non-fibrous components in the surface [22,23].

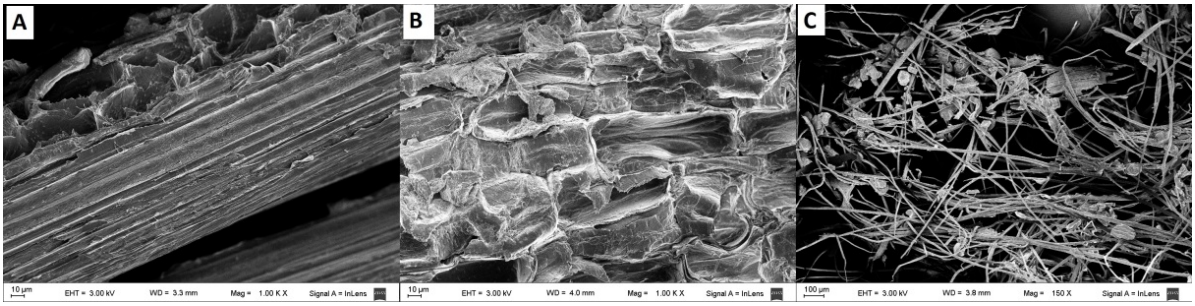

Figure 2: FESEM of **A:** Tacuara Cane; **B:** delignified cane pulp, **C:** bleached pulps

After alkali treatment due to the partial removal of the materials such as lignin, hemicelluloses, pectin, wax and other impurities, the fiber surface becomes rougher as shown in **2B**. The effect of the subsequent bleaching treatment was evident from the comparison of micrographs **2B** and **2C**, where is observed that the cane fibers bundles separate into individual fibers. This decrease indicates that under the strong chemical treatment conditions almost all the components that bind the fibril structure of the cane were removed thus enabling the fibers to separate into an individual form. This is further indication that the alkali treatment was unable to eliminate all the non-cellulosic components [24]. This is why the bleaching process allows the removal of the remaining non-cellulosic portion in the sample.

3.1.3 ATR-FTIR Spectroscopy Analysis

FTIR spectroscopy was used to verify that the lignin and hemicellulose have been removed during the chemical treated at the Tacuara Cane. In Fig. 3 is shown the FTIR spectra of the original Tacuara Cane (**A**), delignified fibers (**B**) and bleached cellulose (**C**). All spectra showed a broad and strong absorption band centered at about 3340 cm^{-1} attributed to stretching vibrations of O-H bond resulting from of the hydroxyl groups present in the structure and the weak peak centered at 2913 cm^{-1} attributed to C_{sp3}-H stretching, meanwhile the signal at 1019 cm^{-1} was due to C-O-C stretching from pyranose ring [17,25], in addition we observed the C-O stretching characteristic of lignin [26]. In A spectrum is observed between 1600-1800 cm^{-1} characteristic signs of stretching C=O from lignin residues, these signals decrees with chemical treatments (see **B** and **C** spectra). It is important to note that the bands located around 1644 cm^{-1} had a decreasement attributed to functional group of acetyl and ester to present in hemicellulose [27]. The same effect is observed in the peaks around 1521 and 1308 cm^{-1} belonging to symmetry and asymmetry stretching of the aromatic ring present in lignin. These signals disappeared in the spectrum of the purified cellulose [26].

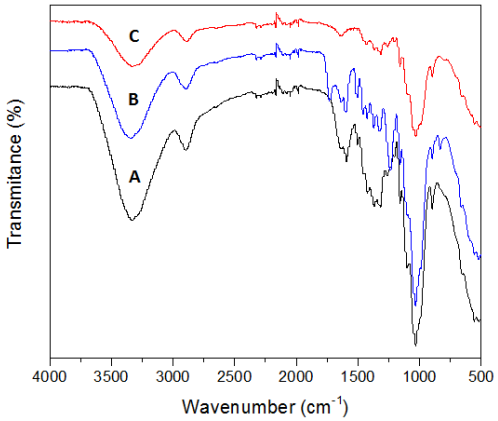

Figure 3: FTIR spectra of **A:** Tacuara Cane; **B:** delignified fibers with NaOH, **C:** bleaching cellulose

3.2 Characterization of Cellulose Nanofibers

3.2.1 FESEM Observations Cellulose Nanofibers

Fig. 4 FESEM shows the nanofibers obtained after the mechanical-ultrasonic process by MASUKO®. Through this process, defibrillation is achieved obtaining nanometric fibers as seen in Fig. 4 A and B from the yellow circle, forming a web-like-structure. Since Fig. 4(C), it can observed nanofibers about 20 nm in diameter.

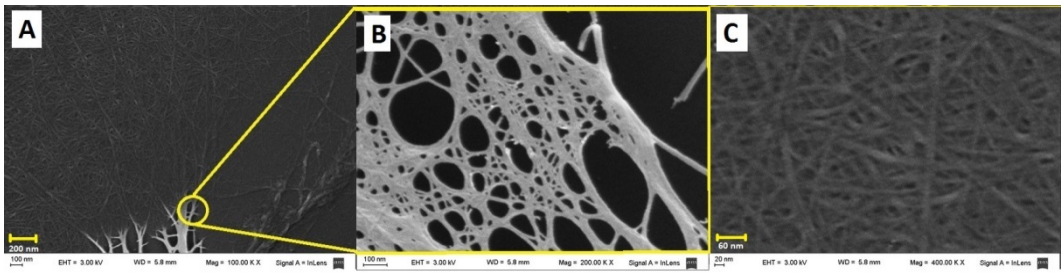

Figure 4: FESEM of **A**, **B**, **C.** CNF Tacuara Cane, with different magnifications, **A:** 100.00KX; **B:** 200 KX and **C:** 400.00 KX

FESEM imaging can zoom into the nanofibril network to provide clear visualization of ultra-fine morphological development during mechanical fibrillation (Fig. 4C). These interconnected networks of nanofibers could provide great reinforcing capability for composite applications [28,29].

3.2.2 X-Ray Analysis

XRD studies were carried out to analyze the effects of the crystallinity in nanofibers. The XRD graph (Fig. 5), showed that all diffractograms exhibited sharp peaks around 2θ = 18° and 23°, which were believed to represent typical cellulose I form. This indicates that the crystal structure of cellulose was not changed during the chemical treatment [20,30,31].

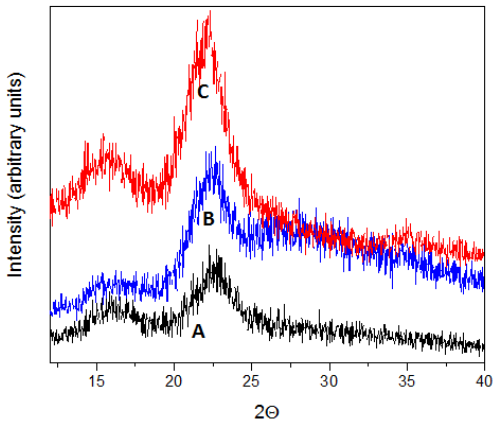

Figure 5: X-ray diffraction patterns of the **A:** Tacuara Cane; **B:** bleached pulps, **C:** CNF Tacuara Cane

The crystallinity of each sample is also calculated and listed in Table 1. In this table increase in the percentages of crystallinity is observed (% CrI), after the cane is subjected to the different chemical and mechanical processes. This crystallinity increase was undoubtedly due to the removal of hemicellulose and lignin, which exist in amorphous regions. This leads to the realignment of cellulose molecules [8,32].

Table 1: Percentage of crystallinity

Sample	CrI(%)
Tacuara cane	48.5
Bleached pulps	52.0
CNF	65.5

3.2.3 Thermostability Analysis

Thermal stability of the extracted cellulose (red line) and NFC (black line), were analyzed by (TGA) and it is showed in Fig. 6. For both samples, the thermal decomposition occurred in three main steps. The first step, recorded below 100°C, corresponds to the moisture evolution of the water absorbed. The second degradation occurs above 150°C until approximately 210°C, resulted mainly from the thermal decomposition of hemicelluloses and some portion of lignin. At temperature range (212-285°C) corresponding to the last degradation was associated with degradation of cellulose and lignin. In this temperature range, the rupture of the glycosidic linkages is generates due to depolymerization of the cellulose. Also, due to the presence of the phenyl groups in the lignin the decomposition is extended to the high temperature range, starting below 200°C and up to 450°C.

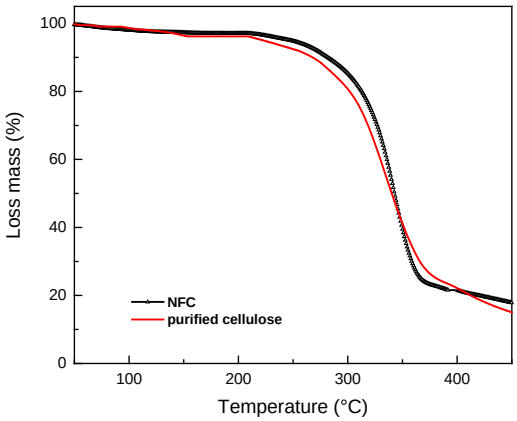

Figure 6: TGA curve of cellulose and cellulose nanofibers from Tacuara cane

On the other hand, the cellulose showed a decomposition temperature at 306°C, while for the CNF it was 314°C. The better thermal stability of nanocellulose could be attributed to the removal of amorphous regions of cellulose and residual lignin as well as its higher degree of crystallinity [8,15]. This result is also consistent with the results obtained from the XRD and FTIR, indicating that the chemical and mechanical process did not influence cellulose chemical composition, crystal structure, and thermostability.

Finally, with regard to the analysis carried out by DSC, a transition in 53.3°C was observed during cooling, as reported by Szczesniak et al. (2008), this temperature is distant from the decomposition temperature, these facts make the material interesting, which makes it an excellent candidate to be used in the formulation of polymer matrices that allows to improve its thermal and mechanical characteristics.

4 Conclusion

In this work we show the use of the endemic plant of Argentina to increase the commercial value, in this case we obtain CNF capable of being used in different industrial applications. The characterization allowed us to confirm the purity of the CNF, due to the elimination of the other components present using a new green methodology. It is important to note that this NFC recovery gives added value to an invasive plant in the delta that is being discarded due to its endemic character. Considering the high thermal stability of the cellulose nanofibers of Tacuara Cane can serve to know their applicability as biocomposites processing. It is important to take into account the possibility of using it as an effective reinforcement material in packaging or coating film.

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