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Research Article

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Posted Date: June 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-552986/v1>

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Micro/nanostructured lignonanocellulose obtained from steam-exploded sugarcane bagasse

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Abstract

This work demonstrates the isolation of lignocellulose micro-nanofibrils, nanocrystals and nanospheres from steam-exploded sugarcane bagasse (SEB). First, steam-explosion was carried out in sugarcane bagasse at 195 °C for 7.5 and 15 min (SEB-7.5 and SEB-15). Untreated sugarcane bagasse was also pretreated by mechanical refining (MRB) in a PFI mill for comparison. Then, SEB and MRB fibers were submitted to alkaline delignification, hypochlorite bleaching for 60 or 180 min (HB60 and HB180), enzymatic hydrolysis (endoglucanase), and high-pressure homogenization. The resulting materials were characterized regarding chemical composition by high-performance liquid chromatography, width and morphology by transmission electron microscopy, crystallinity by X-ray powder diffraction and aspect ratio (length/diameter) by rheology. Levels of glucans (mostly cellulose), hemicelluloses and lignin were: 53.8%, 19.7% and 11.6% in MRB-HB180; 87.8%, <0.1% and 7.2% in SEB-7.5-HB60; 84.3%, <0.1% and 4.8% in SEB-7.5-HB180, 85.9%, <0.1% and 8.4% in SE-15-HB60; and 82.2%, <0.1% and 5% in SE-15-HB180, respectively. Aspect ratios and crystallinity indexes were similar among all groups (~100 and ~73%, respectively), except for SE-15-HB180 (~78.5 and 77%, respectively). MRB-HB180 and SE-15 (HB60 and HB180) had the lowest fiber widths (<20 nm) with the presence of nanocrystals in SE-15-HB180, while in the SE-7.5 (HB60 and HB180), fiber widths were greater than 50 nm with the presence of lignin nanospheres in SE-7.5-HB60. Hence, lignocellulose nanomaterials with aspect ratios ranging from micro/nanofibers to nanocrystals were isolated from SEB using a cost-effective production process.

Keywords: sugarcane bagasse, steam explosion, delignification, enzyme-assisted high-pressure homogenization, lignonanocellulose.

Introduction

Worldwide agro-industrial activities generate substantial amounts of residual waste streams every year. Notwithstanding the fact that these residues are often originated from non-toxic resources, their improper disposal may cause environmental pollution and a severe impact on human health. One common industrial destination that avoids solid waste disposal is its incineration to produce bioelectricity or combined heat and steam, which must be carried out under strict control to reduce greenhouse gas emissions as much as possible. In general, energy savings in the range of US\$ 200 to 400 can be achieved for every 1 ton of burnt wastes (dry basis) (Mathur and Srivastava 2019). By contrast, cellulosic crop residues can be utilized for the isolation of nanocelluloses, whose selling price is in the range of US\$ 2,000-20,000 ton⁻¹ (Assis et al. 2017; Assis et al. 2018). Nanocelluloses, some of which already industrially available, are of special interest due to their high added-value and broad spectrum of applications. Although nanocellulose biocompatibility and biodegradability are key for applications in sustainable products, technological developments are still lacking to accommodate a cost-effective production process, adapted to local resources and to the type of feedstock available as raw material. Further, most of the commercially available nanocelluloses come from bleached chemical pulps, which leads to a low yield of the whole operation.

Cellulose nanofibers, defined as nano-objects with width in nanoscale (3-30 nm), are divided into two types: cellulose nanofibers (CNF) containing both crystalline and amorphous regions with aspect ratio (length/width) usually greater than 50, and cellulose nanocrystals (CNC) containing predominantly crystalline and paracrystalline regions with aspect ratio usually lower than 50 and greater than 5, not exhibiting longitudinal splits, inter-particle entanglement, or network-like structures (Kumagai et al. 2019; Mariano et al. 2014). Mainly due to their excellent physical-chemical (*e.g.*, non-toxic, biocompatible, anisotropic, and high in surface area) and mechanical properties, nanocellulosic materials have gained industrial attention for a wide range of applications, such as in the biomedical, food, cosmetics, construction, automotive and aerospace industries (Hiloidhari et al. 2020; Huang et al. 2019; Wu et al. 2019).

Sulfated-CNC and oxidated-CNF (*e.g.*, Celluforce® and Nippon®, respectively) are examples of commercially available nanocellulosic materials. Sulfated-CNC are obtained by hydrolysis of bleached cellulose using 50 to 60 wt% dilute sulfuric acid at 50 to 60 °C. After neutralization and several washing steps, the resultant CNC possesses high colloidal stability due to its anionic surface (R-OSO₃⁻ with zeta potential of -30 to -60 mV at pH 7.0) (Pirich et al. 2019). Hence, sulfated CNC can be easily dispersed in water even after spray-drying due to electrostatic repulsion. On the other hand, these materials have a relatively low thermostability, from ~300 °C for bleached cellulose or non-functionalized CNC to ~160 °C for sulfated CNC (Nan et al. 2017), which limits application in processes such as thermal extrusion (*e.g.*, blends with polyamides at 270 °C) (Sucharitpong et al. 2020). Oxidated-CNF are obtained by a pre-oxidation step of bleached cellulose using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) as catalyst in alkaline medium (pH 10). After neutralization, the C6-carboxylated fiber surface allows defibrillation to the nanoscale dimensions (diameters < 100 nm) by mechanical treatment (*e.g.*, microgrinding and high-pressure homogenization) with considerable energy savings, from > 200 kW h⁻¹ kg⁻¹ to ≤ 1 kW h⁻¹ kg⁻¹ (Zhou et al. 2018).

The main problems regarding both methodologies are the use of environmentally hazardous chemicals, the requirement of high-cost corrosion-resistant equipment, and the need for remediation of side reaction products such as sulfuric acid. Side products such as fermentable sugars could be further used for biofuels production and this may represent an important contribution to the process economics (Arantes and Saddler 2011; Chinga-Carrasco et al. 2019; Xie et al. 2018).

An interesting alternative for CN production is the auto-hydrolysis of lignocellulosic materials by steam explosion (SE), a technique that is well-established at industrial scale for cellulosic ethanol production. SE consists in pretreating biomass with saturated steam at high pressures and high temperatures by short residence times, followed by the abrupt

decompression of the reaction vessel causing the explosion effect (Bhatia et al. 2019). During this process, the high-pressure steam penetrates the biomass structure, releasing its latent heat of vaporization. With this, acetic acid is released from the acetyl groups of hemicelluloses, promoting their partial hydrolysis into oligomers and monomers, depending on pretreatment severity. Lignin is coalesced and redistributed in small particles, while cellulose is affected to a much lesser extent, being normally recovered in high yields (Bhatia et al. 2019). With this, biomass recalcitrance is partially compromised, and pretreated materials are made more accessible to physical, chemical and biological conversion. Also, the explosion effect imposes a significant physical stress to the already softened lignocellulosic material, helping the breakdown of fiber-to-fiber bonding, and leading to a significant increase in both substrate porosity and surface area. The final product is composed of a thick fiber slurry that generates water-soluble and water-insoluble fractions by filtration. The first is mainly composed of hemicellulose sugars (C5 stream) and dehydration by-products, while the latter contains lignified cellulosic fibers that could be used for nanocellulose production after delignification.

The minimum selling price of commercial oxidated-CNF ranges from USD 1,893 to 2,440 ton⁻¹ (Assis et al. (2018), while that of sulfated-CNC ranges from USD 5,900 to 7200 ton⁻¹ (Assis et al. (2017). On the other hand, Metgen® recently estimated the cost of CNC production in € 500 to 800 ton⁻¹ when produced as a biorefinery side streams (Metgen 2021). As most of these processes are based on bleached kraft pulps, the development of nanocellulose isolation from agricultural residues could represent a huge reduction of the final product cost. Furthermore, CN extracted from pretreated lignocellulosic materials may retain moderate lignin contents, rendering nanomaterials with interesting physical and chemical properties. Nanocelluloses containing lignin or lignified nanocelluloses (LNC) are considerably less hydrophilic than lignin-free nanocelluloses, and this may improve their interaction with hydrophobic materials as well as their drying, transport and storage performances (Deepa et al. 2019; Gu et al. 2019; Silva et al. 2019).

Due to the strong association that exists in lignin-carbohydrate complexes, high pretreatment severities (*e.g.*, high temperatures and residence times, use of exogenous acid catalysts, and application of high shearing strengths in mechanical treatments) and delignification (*e.g.*, alkaline washing, solvent extraction, and oxidative bleaching) are needed to obtain nanomaterials with desirable physical and chemical properties (Kumar et al. 2014; Zhou et al. 2018). The presence of lignin impairs the isolation of nanoscale particles because it acts as a lubricant that reduces shearing during mechanical refining. However, Rojo et al. (2015) demonstrated that partial delignification, from 30-40 wt% to 7-11 wt% lignin contents, was already enough to improve the micro-grinding performance to produce lignocellulose nanofibers (LCNF). The presence of relatively low lignin contents in biomass, although

requiring higher mechanical energy for grinding, produced nanofibers with narrower diameter distributions. Another detrimental effect of lignin is enzyme adsorption when endoglucanases are used to facilitate mechanical refining by weakening fiber strength and fiber-to-fiber bonding (Han et al. 2021; Lu et al. 2018). In addition, Pereira and Arantes (2020) carried out the enzymatic hydrolysis of bleached steam-exploded sugarcane bagasse (SEB) to obtain a soluble fraction with high sugar concentration ($> 120 \text{ g L}^{-1}$ glucose) and an insoluble fraction with CNCs with 20 nm in diameter.

In this work, the extraction of LNC from partially bleached SEB was evaluated. The variables explored in the reaction were steam explosion residence times of 7.5 and 15 min at 195°C and sodium hypochlorite (NaOCl) bleaching times of 60 and 180 min. All resulting materials were pre-hydrolyzed with endoglucanases followed by mechanical treatment with high-pressure homogenization. LNC with different aspect ratios were obtained with characteristics of lignocellulose nanocrystals (LCNC) and LCNF by varying steam explosion and bleaching times. These results demonstrated that lignin-containing cellulosic nanomaterials with interesting and tailored properties and characteristics could be successfully obtained from steam-exploded sugarcane bagasse.

Materials and Methods

Materials

Sugarcane bagasse was donated by Raízen® (Piracicaba, SP). Other reagents were acquired in analytical grade from Sigma-Aldrich®. Standards (glucose, xylose, arabinose, acetic acid, furfural and 5-(hydroxymethyl)furfural), solvents, and reagents for chromatographic and spectrometric analyses were acquired at the specification required by the corresponding analytical procedures. The endoglucanase (Novozymes 476) used for hydrolysis was supplied by Novozymes® (Bagsværd, Denmark). A simplified diagram of the experimental approach is illustrated in Figure 1.

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Steam explosion by auto-hydrolysis

Sugarcane bagasse (50 wt.% moisture content) was pretreated by steam explosion in a 10-L stainless steel reactor connected to a high-pressure steam boiler and equipped with sensors to monitor temperature and reaction time. The reaction conditions were based on the preliminary studies of Pitarelo et al. (2016), using 195 °C for reaction times of 7.5 and 15 min. After this, the pretreated material was released from the pressure vessel into a stainless-steel cyclone where it was collected from with a moisture content of 75-80 wt.%. Then, the pretreatment slurry was centrifuged to separate the pretreated fibers from the water-soluble acid hydrolysate (C5 stream). The solid fraction was washed with water at 10 wt.% water-insoluble solids (WIS) for 60 min under mechanical stirring (150 rpm) at room temperature (~25 °C). The fiber slurry was centrifuged once again, and the solid fraction was stored in vacuum-sealed plastic bags at 4 °C. Steam-exploded samples derived from residence times of 7.5 and 15 min were labeled with the acronyms SEB-7.5 and SEB-15, respectively.

Mechanical refining

Mechanical refining was used as a reference to evaluate the impact of steam explosion on sugarcane bagasse structure and composition. Sugarcane bagasse was first processed in a Sprout Waldron refiner and further refined by 4,000 revolutions at 10 wt.% in a PFI mill. The resulting material was labeled as mechanically refined bagasse (MRB).

Delignification

Alkaline delignification was carried out on both steam-exploded and mechanically refined sugarcane bagasse according to Rocha et al. (2012). Briefly, fibers were suspended in aqueous 0.3 mol L⁻¹ NaOH to reach 5 wt.% WIS. The suspension was heated up to 80 °C and kept under vigorous and constant stirring for 60 min. Then, the reaction was quenched by a 10-fold dilution with tap water at room temperature. The solids were recovered by centrifugation and washed several times with tap water until pH 7.0.

After alkaline delignification, samples were suspended in 0.05 mol L⁻¹ NaClO solution to reach 5 wt.% WIS. The suspensions were heated up to 70 °C and kept under vigorous and constant stirring for 60 or 180 min. For the 180 min bleaching procedure, a new batch of fresh aqueous NaClO was added at every 60 min. Then, the reactions were quenched with 10-fold tap water volume at room temperature (25 °C). The solids were recovered by centrifugation and washed several times with tap water until pH 7.0.

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with an equivalent of 320 g of Novozyme 476 (Novozymes®) per ton of bleached pretreated sugarcane bagasse (MRB or SEB). The reaction was performed at 50 °C and 2.5 wt.% WIS in sodium acetate buffer (0.05 mol L⁻¹) pH 4.5 as previously described by Tarrés et al. (2016). After 180 min, the reaction was quenched by heating at 80 °C for 10 min followed by addition of 3 g L⁻¹ NaOH (2.5 wt.% WIS) at constant stirring. Then, the suspension was centrifuged, and the solids were washed exhaustively with tap water until pH 7.0.

High-pressure homogenization

The suspended solids were adjusted to 1 wt.% WIS after enzymatic hydrolysis using tap water, and mechanically processed through a high-pressure homogenizer (PANDA Plus 2000 homogenizer – Gea Niro Soavi, Italy) for a total of 9 passes (3 at 300 bar, 3 at 600 bar, and 3 at 900 bar) according to the methodology described by Tarrés et al. (2016). The resulting fiber suspension was kept at 4 °C until analysis.

Chemical composition analysis

Lignocellulosic materials were analysed for their chemical composition using the recommendations of the National Renewable Energy Laboratory (NREL, Golden, USA). Moisture contents were determined in an infrared balance at 105°C until constant mass. Ash contents were determined in a muffle at 575 °C for 4 h according to the NREL/TP-510-42622 method. Total extractives were obtained from a two-stage extraction system in a Soxhlet apparatus, first with water (16 h) and then with ethanol 95% for 24 h according to the NREL/TP-510-42619.

Total lignin and carbohydrate contents were determined after dilution sulfuric acid hydrolysis following the NREL/TP-510-42618 method. Samples were initially treated with 72% H₂SO₄ (v v⁻¹) for 60 min at 30 °C. Then, the material was diluted to 3% H₂SO₄ (v v⁻¹), conditioned at 121 °C for 60 min and filtered through a medium porosity sintered glass crucible. Acid-insoluble lignin was determined gravimetrically, while acid-soluble lignin was quantified by ultraviolet spectroscopy at 240 nm. Carbohydrates and their corresponding dehydration by-products were determined in the resulting acid hydrolysates by high-performance liquid chromatography using a Shimadzu HPLC model LC10AD (Kyoto, Japan). The system was equipped with a SIL10A autosampler, a DGU 14A mobile phase degasser, a CTO 10A heating oven, and a RID10A refractive index detector. Analyses were carried out at 65 °C in a Hi-Plex H column (Agilent, 300 x 7.7 mm) preceded by a Hi-Plex H⁺ column (Agilent, 50 x 7.7 mm),

which were eluted with 5 mmol L⁻¹ H₂SO₄ at a flow rate of 0.6 mL min⁻¹ (Pereira Ramos and Domingos Fontana 2004). Quantification was based on calibration curves that were build using primary standards for cellobiose, glucose, xylose, arabinose and acetic acid in the 0.05 to 2 mg mL⁻¹ concentration range. Mass balance calculations considered the loss of one water molecule during hydrolysis, generating correction factors that corresponded to 0.95 for cellobiose, 0.90 for glucose, 0.88 for xylose and arabinose and 0.70 for acetic acid.

Transmission Electron Microscopy

Morphological analyses and dimension measurements after high-pressure homogenization were carried out by transmission electron microscopy (TEM). Samples (5 µL of 10 µg mL⁻¹ fiber suspensions) were deposited in formvar grids coated with carbon. After 60 min, the excess liquid was absorbed with a filter paper strip and the sample was dried at 25 °C for 48 h. Analyses were performed in Jeol JEM-1400 Plus TEM instrument operating with an electron acceleration voltage of 100 keV, and images were recorded with a TVIPS F216 TemCam camera (2040 x 2040 px). Fiber measurements were carried out using the ImageJ software.

Rheology

Rheological properties of LNC suspensions were measured using a Thermo Scientific Haake Rheostress 1 rheometer (Karlsruhe, Germany) that was equipped with cone and plate geometry sensor (40 mm in diameter, cone 2°). The gap between the plates was 1 mm at 25 °C. Steady-state viscosity measurements were carried out in a range of shear rates from 0.01 to 1000 s⁻¹. Changes in viscosity were recorded in nanofiber suspensions at the following concentrations: 1.0, 2.5., 5.0 and 10 mg mL⁻¹. Viscosity values at the shear rate of 1000 s⁻¹ were used to calculate both specific (η_{sp}) and relative (η_{rel}) viscosities using Equations 1 and 2 (Huggins 1942; Kraemer 1938),

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} \quad (\text{Eq. 1})$$

$$\eta_{rel} = \frac{\eta}{\eta_0} \quad (\text{Eq. 2})$$

where η is the suspension viscosity at the shear rate of 1000 s⁻¹ and η_0 is the viscosity of deionized water. Then, these values were plotted against the sample concentration (c) using Equations 3 and 4, respectively,

$$\frac{\eta_{sp}}{c} = [\eta] + k_1[\eta]^2 c \quad (\text{Eq. 3})$$

$$\frac{\ln \eta_{rel}}{c} = [\eta] + k_2[\eta]^2 c \quad (\text{Eq. 4})$$

were k_1 is the Huggins's constant, k_2 is the Kraemer's constant and $[\eta]$ is the intrinsic viscosity. Real $[\eta]$ values were calculated using the average of the two calculated intercepts.

The aspect ratios (f) of LCNF (length/diameter relationship) were predicted from the $[\eta]$ values according to Equation 5 (Simha 1940). Hence, the Simha's plot was used to correlate the achieved $[\eta]$ values with their corresponding f values.

$$[\eta] = \frac{f^2}{15 (\ln 2f - 1.5)} + \frac{f^2}{5 (\ln 2f - 0.5)} + \frac{14}{15} \quad (\text{Eq. 5})$$

X-ray powder diffraction

X-ray diffraction (XRD) measurements were performed in a Shimadzu XRD 6000 diffractometer that was operated at 40 kV and 30 mA with Cu K α radiation ($\lambda = 0.15418$ nm). Samples were lyophilized, placed on aluminum stubs, and measured to estimate crystallinity as described by Chen et al. (2007). Data were collected in the reflectance mode in the 2θ range of 10-40° at 0.5 °min⁻¹. Diffractograms were adjusted with the Gaussian model (Fityk 0.8.0) and the crystallinity index (CrI, %) was calculated using Equation 7 (Hermans et al. 1946),

$$CrI (\%) = 100 \left(\frac{A_c}{A_t} \right) \quad (\text{Eq. 7})$$

where A_c corresponds to the total crystalline area and A_t is the total area (amorphous and crystalline) under the diffractograms.

Results and discussion

Chemical characterization of pretreated cane bagasse

The chemical composition of the starting material is given in Table 1. In average, sugarcane bagasse contained 40.6% glucans, 21.6% xylans, 21.0% total lignin (acid-soluble plus acid-insoluble insoluble lignins), 4.2% extractives and 2.8% ashes. This composition agrees with other reports found in the literature, in which sugarcane bagasse was characterized using the same analytical methods (Carrasco et al. 2010; Moraes Rocha et al. 2011; Silva et al. 2010; Szczerbowski et al. 2014). Nonetheless, small variations are expected in these values due to factors such as sample origin, presence of impurities, and impact of cultivation conditions, as well as differences in the technologies used for harvesting and sugarcane processing.

Table 1. Chemical composition of native (SB), mechanically refined (MRB) and steam-exploded (SEB) sugarcane bagasse before and after alkaline delignification (AD) and alkaline delignification followed by hypochlorite bleaching for 60 (HB60) and 180 min (HB180).

Components (%)	SB	MRB		SEB for 7.5 min at 195 °C				SEB for 15 min at 195 °C			
		AD	HB180	SEB7.5	AD	HB60	HB180	SEB15	AD	HB60	HB180
Glucans	40±1	53±1	54±1	56±1	77±1	87±1	84±1	49±1	79±1	84±1	82±2
Xylans	22±1	18.4±0.3	19±1	3.4±0.2	2.9±0.1	b.d.l.	b.d.l.	2.0±0.3	1.4±0.1	b.d.l.	b.d.l.
Arabinosyl residues ²	1.57±0.05	1.5±0.1	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Acetyl groups ³	2.8±0.1	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.
Total lignin	21.0	12.3	11.6	32.5	8.2	7.2	4.8	41.5	8.3	6.3	5.0
Acid-soluble	0.02±0.01	2.1±0.2	5.6±0.1	0.5±0.1	1.1±0.1	2.5±0.1	2.1±0.1	0.5±0.1	1.1±0.1	2.1±0.1	2.0±0.5
Acid-insoluble	21±1	10.2±0.1	6.0±0.2	32±1	7.1±0.3	4.7±0.2	2.7±0.2	41±1	7.2±0.5	4.2±0.5	3.0±0.5
Ashes	2.8±0.3	8.1±0.6	5.5±0.5	3.2±0.1	7.3±0.2	3.1±0.1	7.2±0.3	4.2±0.5	7.6±0.6	4.2±0.5	8.9±0.3
Total extractives	4.2±0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Water-soluble	2.3±0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethanol-soluble	1.9±0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total (%)	94.4	93.3		95.1	95.4	97.3	96.0	96.7	96.3	94.5	95.9
Mass yield (%)	100	65±4	54±3	59±4	n.d.	33±4	31±5	57±6	n.d.	32±4	30±5

b.d.l., below the detection limit of the method; n.d., not determined;
¹ Measured as xylose in acid hydrolysates;
² Measured as arabinose in acid hydrolysates and present as substituents of the xylan backbone;
³ Measured as acetic acid in acid hydrolysates and present as substituents of the xylan backbone.

Sugarcane bagasse was subjected to different pretreatment conditions prior to high-pressure homogenization to produce nanocellulose. Steam explosion is a pretreatment that is well-known to remove most of the hemicellulose component from biomass, resulting in steam-treated materials primarily composed of glucans (mostly cellulose) and lignin (Martino et al. 2017; Zhang et al. 2007). On the other hand, mechanical refining is not supposed to affect biomass chemical composition, but some ashes and/or extractives (< 1%) may be removed by cold water during Sprout Waldron defibrillation. Hence, after mild alkaline delignification and hypochlorite bleaching, enzymatic hydrolysis and high-pressure homogenization, SEB and MRB rendered bleached nanofibers with different hemicellulose contents (Agbor et al. 2011; Hendriks and Zeeman 2009). Delignification was required in this study because lignin interferes with enzymatic hydrolysis and impairs the effects of high-pressure homogenization during nanocellulose production (Lu et al. 2018; Rojo et al. 2015).

Alkaline delignification of MRB led to a 65.1% recovery of dry biomass. The resulting material exhibited lower hemicelluloses and lignin contents (Table 1), with 86% of glucans being retained in the alkali-insoluble material. During this process, lignin undergoes partial depolymerization and fragments are released to be solubilized in alkali in the form of phenolates, which are subsequently removed by filtration and washing. The recovered solids showed an increase in the ratio between acid-soluble lignin and acid-insoluble lignin, probably due to the reprecipitation of depolymerized fragments that were not removed even after extensive water washing. Acetyl groups were no longer detected after alkaline delignification, while the hemicellulose content (mostly xylans) decreased from 21.7 to 18.4%. Also, there was an increase in the ash content of alkali-insoluble materials because sodium ions (Na^+) were retained as salts by acidic functional groups that are found in both hemicelluloses and lignin. Hydroxyl groups possess high affinity for Na^+ (Budtova and Navard 2016) and can only be removed by ion exchange under acidic conditions. Also, residual lignin contains additional weak acid sites (carboxyl groups and phenolic acids) that would help retaining Na^+ by electrostatic interaction.

Hypochlorite bleaching for 60 min (MRB-HB60 in Table 1) was not enough to impart significant changes to the chemical composition of MRB fibers. By contrast, MRB bleaching for 180 min (MRB-HB180) improved fiber homogeneity, removed chromophores and reduced the hemicellulose content to undetectable levels, but its effect on delignification was not high due to fiber recalcitrance and to the relatively mild conditions applied in this process. Bleaching for 180 min resulted in a total mass loss of 46 wt.% in relation to the starting material (dry basis). The amount of acid-insoluble lignin was reduced from 10.2% in alkali-washed cane bagasse to 6.0% with a concomitant increase in acid-soluble lignin from 2.1 to 5.6% (Table 1). Once again, xylans were not severely affected, producing holocellulose fractions containing

small amounts of lignin that could be used to produce LNC by partial enzymatic hydrolysis followed by high-pressure homogenization.

Steam explosion of sugarcane bagasse at 195 °C for 7.5 or 15 min gave mass recoveries of 56.4 ± 4.1 % and 57.4 ± 6.2 % (dry basis), respectively (Table 1). As a result, the hemicellulose content of sugarcane bagasse (mostly xylan) decreased from 22% to 3.4 and 2.0 % while acetyl groups and arabinosyl residues were not detectable after pretreatment, suggesting the recovery of undecorated xylan fragments of relatively low molecular mass. Glucan recoveries were 83.1 and 70.3 % for these two reaction times, respectively, revealing much higher sugar losses for pretreatments carried out for 15 min (SEB-15 in Table 1). Total lignin contents in steam-exploded materials increased from 21.0% in untreated sugarcane bagasse to 32.5 and 41.5 % in SEB-7.5 and SEB-15, respectively. These values correspond to total lignin recoveries of 91.9 and 113.4 % in relation to the chemical composition of sugarcane bagasse, indicating that hydrolysis prevailed over condensation reactions at shorter residence times into the steam reactor. By contrast, longer residence times led to the formation of both humins and/or pseudo-lignin, as already reported in the literature (Aarum et al. 2018). Humins are condensed acid-insoluble macromolecular structures that are formed from carbohydrate dehydration by-products at high pretreatment severities, while pseudo-lignin arises from contaminations such as resin oils and fats and from condensation reactions involving cinnamic acid derivatives, organic acids and furan compounds. When present in high amounts, these acid-insoluble extraneous materials behave as acid-insoluble lignin, causing an overestimation in its quantitative analysis. Ash contents increased slightly after pretreatment, and this appeared to be another effect of hemicellulose removal.

Alkaline delignification of both SEB-7.5 and SEB-15 dropped their total lignin content to 8.2 and 8.3 %, respectively, having similar impact on both acid-soluble and acid-insoluble lignin contents. The hemicellulose content in both alkali-insoluble materials was further reduced to 2.9% for SEB-7.5 and 1.4% for SEB-15. This incomplete hemicellulose removal may have been due to the temperature (80 °C) and NaOH concentration (0.3 mol L^{-1}) used for delignification, compared to harsher conditions already described in the literature (Kamali and Khodaparast 2015; Thompson et al. 2001). Both ash and glucan contents were not affected by alkaline delignification and their increase in alkali-insoluble materials was proportional to the decrease of other fiber constituents. Alkaline washing followed by hypochlorite bleaching for 60 and 180 min resulted in an overall mass loss of 67 and 69 % in relation to the dry mass of sugarcane bagasse, respectively. Bleaching of alkali-washed SEB-7.5 for 60 or 180 min produced fibers with total lignin contents of 7.2 and 4.8 %, respectively, and by doubling the pretreatment time to 15 min (SEB-15), alkali-washed hypochlorite-bleached fibers with total lignin contents of 6.3 and 5.0 % were obtained. Hemicellulose contents were again under

undetectable levels, while higher brightness could only be observed after bleaching for 180 min.

Compared to MRB, alkaline delignification and bleaching for 180 min were more effective for SEB, but the resulting bleached materials still contained relatively high total lignin contents (Table 1). However, as already observed for MRB, acid-soluble lignin corresponded to 35-40% of the total lignin content of fully bleached materials (HB180 in Table 1), while for alkaline delignification alone its contribution was much lower, falling the range of 13.2 to 13.4 % of the corresponding total lignin contents.

The steam explosion, alkaline delignification and bleaching procedures described above generated five bleached materials for LNC production. With this, the effects of lignin and hemicellulose contents were accessed on the efficiency of LNC isolation by enzymatic hydrolysis followed by high-pressure homogenization.

Enzymatic hydrolysis of partially delignified fibers

All bleached biomasses were subjected to the same enzymatic hydrolysis condition using a commercial endoglucanase preparation. Enzymatic hydrolysis was used to decrease the fiber-fiber entanglement and consequently reduce the energy required for mechanical defibrillation (Kargarzadeh et al. 2017; Xie et al. 2018). Furthermore, treatment by endoglucanases for short reaction times (180 min) are not expected to result in significant mass losses due to their classical mode of action, which limits reducing sugar release by acting at the more accessible regions of the cellulose supramolecular structure (amorphous cellulose). Then, enzyme-treated fiber suspensions were passed through a high-pressure homogenizer under the same experimental conditions. The presence of nanofibers was indicated by achieving viscous suspensions with a gel-like appearance without evidence for macroscopic aggregates. Also, there was no significant change in chemical composition after mechanical treatment, as indicated in Figure 2.

Figure 2. Chemical composition of sugarcane bagasse compared to lignocellulose nanomaterials that were obtained after mechanical refining or steam explosion (7.5 and 15 min at 195 °C), followed by alkali washing, hypochlorite bleaching (60 and 180 min), enzymatic hydrolysis, and high-pressure homogenization.

Rheological characterization after high-pressure homogenization

Changes in rheological behavior are expected after reduction of fiber diameter from a micrometric to a nanometric scale, forming a gel-like structure in water that is organized by semi-permanent hydrogen bonding networks (Nguyen et al. 2015; Samaniuk et al. 2011). With a decrease in fiber diameter, there is a corresponding increase in surface area and the interaction with water promotes their orientation when exposed to a laminar flow. Hence, the minimum amount of mass required to reach the gel point is considerably reduced. By gathering knowledge about the gel point, one can estimate fiber dimensions by determining their corresponding aspect ratio (Delgado-Aguilar et al. 2015a; Liu et al. 2011; Varanasi et al. 2013).

Accurate fiber dimensions in nanoscale (especially length) are very hard to achieve by electron microscopy because cellulose nanofibers tend to aggregate during sample preparation for analysis. By contrast, rheological measurements and sedimentation techniques are fast and robust alternatives to access the properties of cellulose nanomaterials because

they do not require drying and produce results that are more representative of the entire fiber population (Delgado-Aguilar et al. 2015a; Liu et al. 2011; Varanasi et al. 2013).

The aspect ratio of LNC isolated in this work was first investigated by employing rheological measurements. Viscosity changes at steady state as a function of the shear rate are shown in Figure 3 for 10.0, 5.0, 2.5 and 1.0 mg mL⁻¹ LNC suspensions. Higher concentrations resulted in higher viscosities for all dilutions throughout the shear rate curve, indicating the formation of a more rigid fiber network. Also, sample viscosity decreased with an increase in shear rate, exhibiting a typical shear thinning behavior.

Each shear-thinning curve could be divided in five regions. In region I, fibers orient themselves slowly along the shear direction under low shear force, causing a gradual decrease in viscosity. In region II, the viscosity rate decreases, exhibiting lower changes in viscosity with an increase in shear rate probably due to the formation of a more entangled fiber network. In region III, as the shear rate increases continuously, shear forces cause the tangled network to break into individual nanofibers, leading to a decrease in the system viscosity. In region IV, another plateau appears because most of the entangled network was interrupted, with the formation of a well-oriented nanofibrillar structure. The results described above are similar to those observed by Delgado-Aguilar et al. (2015a) for cellulose nanoparticles in water suspension. LCNF suspensions derived from MRB-HB180 (Figure 3a) showed higher viscosities than those derived from steam-exploded materials (Figures 3b to 3e). This difference in viscosity can be attributed not only to differences in the LCNF aspect ratio, but also to differences in their chemical composition. As discussed before, LCNF from MRB-HB180 had a hemicellulose content higher than that of LCNF from SEB-HB and their greater hydrophilicity may have influenced trends in sample viscosity.

Figure 3. Steady-state viscosity vs. shear rate of (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180 suspensions with concentrations of 1.0 mg mL⁻¹ (black squares), 2.5 mg mL⁻¹ (red circles), 5 mg mL⁻¹ (blue triangles) and 10 mg mL⁻¹ (olive stars) at 25 °C. At low concentrations and low shear rates, viscosity could not be measured precisely due to limitations in the rheometer sensitivity.

To access the LCNF aspect ratio, $\ln \eta_{rel}/c$ and $\ln \eta_{sp}/c$ values were plotted against sample concentration at a shear rate of 1000 s⁻¹ (Figure 4-A to 4-E). With this, the y-axis intercepts from Equations 3 and 4 yielded the corresponding apparent intrinsic viscosities $[\eta]$ and their averages indicated the true $[\eta]$ value, which was 500, 644, 517, 600 and 392 for

LCNF derived from MRB-HB180, SEB-7.5-HB60, SEB-7.5-HB180, SEB-15-HB60 and SEB-15-HB180, respectively.

Figure 4. Values of $\ln \eta_{rel}/c$ and η_{sp}/c and Kraemer and Huggins' plots for ultra-homogenized fibers suspensions: (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180 at a shear rate of 230 s^{-1} at 25°C and (d) Simha's plot used to estimate the aspect ratio (f) of ultra-homogenized fibers.

The plotted values of $[\eta]$ vs. f using the Simha's equation (see Material and Methods for further clarification) are shown in Figure 4f. Simha's plot was presented in the form of a parabola, in which $[\eta]$ gradually increased as f -values increased. By interpolating the values of $[\eta]$ in the Simha's plot, the following f values were obtained: 90.5 for MRB-HB180, 106.0 for

SEB-7.5-HB60, 92.3 for SEB-7.5-HB180, 101.0 for SEB-15-HB60 and 78.5 for SEB-15-HB180. According to the TAPPI WI 3021 standard, all these aspect ratios (f values greater than 50) fall in the cellulose nanofibril category. All members of the SEB group, except SEB15-HB180, presented an aspect ratio slightly larger than that of MRB group, indicating fibers of larger length and/or smaller diameter. The SEB-15-HB180 group showed an aspect ratio significantly lower than all other materials, suggesting the occurrence of fibers of shorter length and/or larger diameter.

Figure 5 shows the TEM micrographs of lignocellulose micro and nanofibrils. In Figure 5a, the observed nanofibers had width values of 11.7 ± 23.5 nm for MRB HB180, 160.0 ± 80.2 nm for SEB-7.5-HB60, 28.0 ± 12.3 nm for SEB-7.5-HB180, 16.3 ± 4.4 nm for SEB-15-HB60 and 16.2 ± 7.3 nm for SEB-15-HB180. Most of the nanofibers appeared as bundles or agglomerates with widths from 10 to 40 nm, except those of the SEB-7.5 group, which had a higher microfiber content. The presence of microfibers in the latter group corroborated the aspect ratio that was obtained by statistical modeling using the Simha's equation (Figure 4). The precise determination of length was complicated because nanofibers were strongly aggregated or twisted, even after the homogenization process. This highly entangled network of micro and nanofibers had a significant influence on their viscoelastic rheological properties in aqueous suspensions. Fibers with reduced length of 100-400 nm and widths around 16 nm were only observed in group SEB-15 (Figures 5d and 5e) and these could be classified as CNC of according to the TAPPI WI 3021 standard.

Figure 5. TEM micrographs of (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180.

TEM analysis allowed to observe the presence of unexpected particles of spherical morphology in the SEB-7.5-HB60 nanofiber suspension. However, sample had to be contrasted with uranyl acetate to improve their visualization (Figure 6). The average diameter of the spherical particles was 85 ± 37 nm. These nanoparticles seemed to have a high adsorption affinity for the nanofibers because they persisted in the sample even after

exhaustive washing with ultrapure water. We hypothesized that these round nanoparticles were coalesced lignin fragments that were produced during steam explosion. Indeed, Jiang et al. (2018) showed that spherical lignin nanoparticles are produced when the fiber diameter of lignified fibers are reduced to the nanoscale by intense mechanical defibrillation.

Figure 6. TEM micrograph of SEB-7.5-HB60 exhibiting agglomerated spherical nanoparticles. Visualization was improved by contrast using uranyl acetate.

The X-ray diffractograms of lyophilized samples after high-pressure homogenization are shown in Figure 7. All samples exhibited four main diffraction peaks around 15.2° , 16.7° and 22.6° , corresponding to the crystallographic planes $1\bar{1}0$, 110 and 200 of cellulose I, respectively. Steam explosion, alkaline delignification, hypochlorite bleaching, enzymatic hydrolysis and mechanical refining did not cause noticeable changes in the position of the diffraction peaks, revealing that these treatments do not alter the crystalline structure of cellulose. However, LCNF materials with slightly higher crystallinity indexes (CrI) were isolated when steam explosion was carried out for longer residence times of 15 min.

Fig. 7. X-ray patterns of high-pressure homogenized samples

The CrI values calculated for MRB, SEB-7.5-HB60, SEB-7.5-HB180, SEB15 HB60 and SEB15 HB180 were 68.5%, 72.7%, 76.2%, 75.8% and 77.0%, respectively. The CrI variation from 68.5% of MRB HB180 to around 76.0% for SEB-HB180 materials suggest that more drastic pretreatment conditions led to a more extensive removal of hemicelluloses and amorphous cellulose mainly by partial acid hydrolysis. Also, the highest CrI value of SEB-15-HB180 corroborates the observation by TEM of cellulose nanoparticles with a typical aspect ratio of CNC.

The dimensions, aspect ratio and crystallinity index of the obtained LCNF, compared to the literature data for nanocelluloses obtained from steam-exploded sugarcane bagasse, are summarized in Table 4. Although these works use steam explosion and the same biomass source, the post-treatment methodology for nanocellulose isolation was different, producing materials with low lignin content (<1%) and some degree of surface derivatization (TEMPO oxidation and esterification with sulfuric acid). No studies were found using steam explosion followed by alkaline delignification, hypochlorite bleaching, enzymatic hydrolysis, and high-pressure homogenization to isolate nanocelluloses with different lignin contents from sugarcane bagasse.

534 Table 4 - Comparison of lignonanocelluloses obtained in this work with data available in the literature

Sample	Biomass	Method	Total lignin (%)	Diameter (nm)	Length (nm)	<i>f</i>	CrI (%)	Ref.
LCMF+LCNF+LNS ¹	Cane bagasse	SE+AD+HB+EH+HPH	7.2	160 ± 80	400 to >1000	106	72.7	This work
LCNF ²		SE+AD+HB+EH+HPH	4.8	28 ± 12	500 to >1000	92.3	76.2	
LCMF+LCNF ³		SE+AD+HB+EH+HPH	8.4	16 ± 4	300 to >1000	101	75.8	
LCNF+LCNC ⁴		SE+AD+HB+EH+HPH	5.0	16 ± 7	100-400	78.5	77.0	
LCNF ⁵		MRB+HB+HPH	11.6	45 ± 23	>1000	90.5	68.5	
CNF	Cane bagasse	SE+HT+H ₂ O ₂ +HSB+UT	--	20-40	--	--	71.2	1
CNC		SE+AD+AH	--	16 ± 4	201 ± 60	13*	81.6	2
CNF		SE+EH+HB+HPH	--	5-10	--	--	68.1	3
LCNF	Wheat straw	AD+TEMPO+MT+EH+HPH	8.9-18.2	11.16-116	139-705	3.9-6.1*	44-53	4
LCNF	<i>Pinus</i> sp TMP	MT+EH+TEMPO	15, 20 & 25	~12	>1000	>83*	--	5
LCNF	Cereal straws	AD+MT+HE+TEMPO+HPH	13.31	6.81-14.52	251-697	36-48*	44.44	6
LCNF	Stone-ground pulp	AD+HB+MT+HPH	2.47-6.47	~35	>1000	>28*	45	7
LCMF+LCNF+LNS	Cane bagasse	OS+AD+HB+MG	0.1, 2.4 & 14.9	32, 27 & 9	--	--	--	8
LCNF	Wheat straw	AE+MT+MF	4, 8 & 16	17, 14 & 13	>1000	>77*	--	9
LCNF	<i>Eucalyptus</i> sp.	MT+TEMPO+MT+HPH	--	13	>1000	>76*	--	10

535 ¹ SEB-7.5-HB60; ² SEB-7.5-HB180; ³ SEB-15-HB60; ⁴ SEB-15-HB180; ⁵ MRB-HB180; TMP, thermomechanical pulp; LCNF, lignocellulose nanofibers; LCMF, lignocellulose microfibrils; LCNC,
536 lignocellulose nanocrystals; LNS, lignonanospheres; CNF, cellulose nanofibers; CNC, cellulose nanocrystals; SE, steam explosion; AD, alkali delignification; HB, hypochlorite bleaching; MT,
537 mechanical treatment; EH, enzymatic hydrolysis; HPH, high-pressure homogenization; HT, hydrothermal treatment; H₂O₂, peroxide bleaching; HSB, high speed blending; UT, ultrasound
538 treatment; AH, acid hydrolysis; OS, organosolv treatment; MG, micro-grinding; MF, micro-fluidization; *f*, aspect ratio; *, estimated aspect ratio from reported data.
539 References: 1, Feng et al. (2018); 2, Sukyai et al. (2018); 3, Saelee et al. (2016); 4, . Espinosa et al. (2017b); 5, Diop et al. (2017); 6, Espinosa et al. (2017a); 7, Delgado-Aguilar et al. (2016);
540 8, Jiang et al. (2018); 9, Gu et al. (2019); 10, . Herrera et al. (2018).

541 Regarding fiber diameter, except for the SEB-7.5-HB60 group, all samples
542 had similar sizes (10-20 nm) compared to the data reported in the literature for both
543 CNC and CNF (Table 4). Specifically for CNF, most studies report diameter and
544 morphology measurements by electron microscopy, with no reference to fiber
545 length. This is because the accuracy of electron microscopy for length
546 measurements is hampered by aggregation and fiber interlacing (Li, Wu et al.
547 2015). In the present work, this problem was solved by performing rheological
548 analyzes to obtain CNF aspect ratios (f). To compare our rheological LCNF aspect
549 ratios with data reported in the literature for other LCNF types, f values were
550 estimated based on reported fiber diameters and average fiber lengths. In general,
551 the estimated f values were lower than that of LCNC derived from SEB-15-HB180
552 (78.5). However, this difference can be explained by the presence of LCNF in this
553 sample and the effect of its higher fiber length on the corresponding f value.
554 Regarding the crystallinity index, all samples presented values (~72 to 77%) close
555 to those reported in the literature (68 to 79%) for both LCNF and LCNC.

556 Intense mechanical treatments are required for defibrillating biomass fibers
557 to the nanoscale and this can be achieved by increasing the number of passages
558 through the mill or the time and pressure used for homogenization (Khalil et al.
559 2014; Teo and Wahab 2020). However, excessive mechanical treatments may
560 cause a dramatic reduction in cellulose CrI (Potulski et al. 2016; Viana et al. 2019).
561 In this work, bleached SEB fibers were defibrillated to nanometric diameters using
562 nine passages through the mill, which is half that reported in the literature for
563 isolating LCNF from a variety of lignocellulosic materials (García et al. 2016;
564 Ghaemi et al. 2019; Hu et al. 2019; Lee et al. 2014). Also, bleached SEB LCNF
565 had CrI values higher than those reported in the literature (*circa* 45% in Table 5).
566 This was probably due to the extent of hemicellulose removal during pretreatment
567 and to the hydrolytic action of endoglucanases over SEB bleached fibers. In
568 addition to LCNF, the presence of LCNC was also observed in both SEB-15-
569 derived nanomaterials, being predominant in HB180. No other work has
570 demonstrated the production of LCNC from SEB under conditions like those
571 applied in this study.

572 LCNFs have some advantages over lignin-free nanocelluloses, such as
573 ease of drying, redispersion and compatibility with more hydrophobic matrices.
574 Besides, its production process may offer significant economic advantages
575 compared to nanomaterials derived from fully bleached pulps. For example, the
576 cost of producing LCNF from *kraft* pulps using enzymatic hydrolysis followed by
577 high-pressure homogenization was estimated at 2000-5000 € ton⁻¹ (Delgado-

578 Aguilar et al. 2016), a very attractive price compared to alternatives already
579 available in a pre-industrial scale such as TEMPO-oxidized CNF, whose
580 production cost was recently estimated at 206,000 € ton⁻¹ (Delgado-Aguilar et al.
581 2015b). Also, the use of agro-industrial wastes to produce LCNF can further
582 improve the process economics. For example, the cost of steam explosion for
583 materials such as sugarcane bagasse is estimated at around 60 € ton⁻¹ while the
584 bleaching costs are below 100 € ton⁻¹ (Cotana et al. 2015). Thus, LCNFs isolated
585 from steam-exploded sugarcane bagasse could be highly competitive with similar
586 products already available on the market.

587

588 **Conclusion**

589 Steam explosion of sugarcane bagasse followed by alkaline delignification,
590 hypochlorite bleaching, enzymatic hydrolysis, and high-pressure homogenization,
591 resulted in the isolation of lignocellulose nanomaterials with aspect ratios ranging
592 from micro/nanofibers to nanocrystals. The high crystallinity index of these
593 nanomaterials suggests good potential for applications as mechanical
594 reinforcement agents. Also, the presence of lignin could be designed by changing
595 parameters such as pretreatment severity and the extent of alkaline delignification
596 or hypochlorite bleaching. With this, a range of alternative applications may be
597 created by improving their interaction with more hydrophobic composite matrices
598 and by providing better properties for surface coating (e.g., hydrophobization).

599

600 **Acknowledgments**

601 The authors are grateful to CNPq (grants 551404/2010-8 and
602 309506/2017-4) for providing financial support to carry out this study, as well as to
603 Novozymes Latin America (Araucária, PR, Brazil) for donating the enzymes used
604 for hydrolysis and to CYTED (Rede Nanocelia) for providing a fellowship to A.M.F.
605 for a short stay at the University of Girona. This work was also financed in part by
606 the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES,
607 Brasil) – Finance Code 001. Authors also wish to acknowledge the financial
608 support of the Spanish Ministry of Economy and Competitiveness to the project
609 NANOPROSOST (reference CTQ2017-85654-C2-1-R). Dr. Marc Delgado-Aguilar
610 is a Serra Húnter Fellow.

611

612 **Declarations**

613 **Conflicts of interest/Competing interests**

614 The authors declare that no conflict of interest is involved in this manuscript and
615 that they do not have any competing interests.

616

617 **Availability of data and material**

618 The authors made all data public in the international repository Zenodo. UdG
619 authors express their commitment to make them public in the UdG repository.

620

621 **Code availability**

622 Not applicable

623

624 **Authors' contributions**

625 Authors' contributions: LPR, MDA and CLP conceived the experimental design.
626 AMF and GROAT carried out pretreatment, isolation and characterization of
627 lignocellulosic materials. CLP performed microscopical, rheological and X-ray
628 diffraction analyses. QT helped in the execution of enzyme treatments and high-
629 pressure homogenization. AMF and CLP wrote the original manuscript draft. LPR
630 and MDA revised the final version of the manuscript.

631

632 **Ethics approval**

633 Not applicable. This study does not involve humans and/or animals.

634

635 **Consent to participate**

636 All authors are authorized by their home institution and agreed to participate in the
637 development of this research and in the preparation of this manuscript.

638

639 **Consent for publication**

640 All authors are aware of the manuscript submission and agree with its publication.

641

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Figures

Figure 1

Flow diagram of the global reactions to obtain nanofibers of cellulose

Figure 2

Chemical composition of sugarcane bagasse compared to lignocellulose nanomaterials that were obtained after mechanical refining or steam explosion (7.5 and 15 min at 195 °C), followed by alkali washing, hypochlorite bleaching (60 and 180 min), enzymatic hydrolysis, and high-pressure homogenization.

Figure 3

Steady-state viscosity vs. shear rate of (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180 suspensions with concentrations of 1.0 mg mL⁻¹ (black squares), 2.5 mg mL⁻¹ (red circles), 5 mg mL⁻¹ (blue triangles) and 10 mg mL⁻¹ (olive stars) at 25 °C. At low concentrations and low shear rates, viscosity could not be measured precisely due to limitations in the rheometer sensitivity.

Figure 4

Values of $\ln \eta_{rel}/c$ and η_{sp}/c and Kraemer and Huggins' plots for ultra-homogenized fibers suspensions: (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180 at a shear rate of 230 s⁻¹ at 25 °C and (f) Simha's plot used to estimate the aspect ratio (f) of ultra-homogenized fibers.

Figure 5

TEM micrographs of (a) MRB-HB180, (b) SEB-7.5-HB60, (c) SEB-7.5-HB180, (d) SEB-15-HB60 and (e) SEB-15-HB180.

Figure 6

TEM micrograph of SEB-7.5-HB60 exhibiting agglomerated spherical nanoparticles. Visualization was improved by contrast using uranyl acetate.

Figure 7

X-ray patterns of high-pressure homogenized samples

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