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Nanostructure variability of cellulose from plants and the impact on
cellulose nanocrystals production

Variabilidade nanoestrutural de celuloses vegetais e o seu impacto
na produção de nanocristais de celulose

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na produção de nanocristais de celulose

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Dedication

I dedicate this work to my beloved family, my mother Cirena, my father Darci and my brother Marcos. They stay by my side all these years, physically, emotionally, and mentally. My family support helps me to overcome all the difficulties, all these years of work. The patience, kindness and love they gave were the source of my motivation. They are the source of my inspiration, motivation and hopes for a new tomorrow. So, I dedicate this work to them, my beloved family, thank you for everything.

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Abstract

OLIVEIRA, M. M. Nanostructure variability of cellulose from plants and the impact on cellulose nanocrystals production. 2018. 113 p. Tese (Doutorado em Ciência e Engenharia de Materiais) – Escola de Engenharia de São Carlos, Universidade de São Paulo, 2018.

This work investigates the compositional and nanostructural variability of celluloses isolated from plants and the impact of the variability in the production of cellulose nanocrystals. A variable set of cellulose isolated from plants were generated starting with a range of feedstocks (coconut fiber, sisal fiber, eucalyptus sawdust, pine sawdust, sugarcane rind and sugarcane pith), applying a range of cellulose isolation processes (acetosolv, liquid hot water, alkaline, and liquid hot water + alkaline) and adding commercial cellulose (eucalyptus kraft pulp, dissolving pulp, and microcrystalline cellulose) as reference materials. The nanostructural characteristics were evaluated by calorimetric thermoporometry, X-ray diffraction, and moisture sorption isotherms. Composition was evaluated by standard wet chemical analysis and insights on functional groups were obtained by infrared spectroscopy. The cellulose nanocrystals were produced by acid hydrolysis with sulfuric acid and characterized by atomic force microscopy and X-ray diffraction. The measured parameters of the isolated celluloses were spread, showing we could achieve a highly diverse set of substrates. Significant correlations between measured variables across the sample set, indicating possible unforeseen multivariate relations among cellulose features. For example, we could show that cellulose monolayer hydration is determined by both hemicelluloses content (compositional parameter) as well as cellulose crystal width (structural parameter). Cellulose nanocrystals were successfully produced, although in some cases such as for the acetosolv pulps the acid conditions were too aggressive and oxidized the substrates. Finally, some quantitative correlations were seen between the parameters of cellulose substrates and the resulting cellulose nanocrystals. These results supply the first hints about how the nanostructural variability of isolated cellulose can influence the cellulose nanocrystals produced from them.

Key words: Cellulose, variability, nanostructure, nanocellulose, cellulose nanocrystal, biomass.

Resumo

OLIVEIRA, M. M. Variabilidade nanoestrutural de celuloses vegetais e o seu impacto na produção de nanocristais de celulose. 2018. 115 p. Tese (Doutorado em Ciência e Engenharia de Materiais) – Escola de Engenharia de São Carlos, Universidade de São Paulo 2018.

Este trabalho investiga a variabilidade composicional e nanoestrutural de celuloses isoladas de plantas e o seu impacto na variabilidade na produção de nanocristais de celulose. Um conjunto variável de celuloses isoladas de plantas foi gerado a partir de uma série de matérias-primas (fibra de coco, sisal, serragem de eucalipto, serragem de pinheiro, casca de cana e miolo de cana), aplicando uma série de processos de isolamento de celulose (hidrotérmico, alcalino, hidrotérmico + alcalino e acetosolve) e adicionando celuloses comerciais (polpa kraft de eucalipto, polpa para dissolução e celulose microcristalina) como materiais de referência. As características nanoestruturais foram avaliadas por termoporometria calorimétrica, difração de raios X e isotermas de sorção de umidade. A composição foi avaliada por análise química húmida padrão e os conhecimentos sobre grupos funcionais foram obtidos por espectroscopia de infravermelhos. Os nanocristais de celulose foram produzidos por hidrólise ácida com ácido sulfúrico e caracterizados por microscopia de força atômica e difração de raios-X. Os parâmetros medidos das celuloses isoladas foram distribuídos, demonstrando que poderíamos alcançar um conjunto altamente diversificado de substratos. Correlações significativas entre as variáveis medidas foram observadas em todo o conjunto amostral, indicando possíveis relações multivariadas imprevistas entre as características da celulose. Por exemplo, poderíamos demonstrar que a monocamada de hidratação de celulose é determinada tanto pelo conteúdo de hemiceluloses (parâmetro de composição) quanto pela largura do cristal de celulose (parâmetro estrutural). Os nanocristais de celulose foram produzidos com sucesso, embora em alguns casos, como nas polpas acetosolve, as condições ácidas fossem muito agressivas e oxidassem os substratos. Finalmente, algumas correlações quantitativas foram observadas entre os parâmetros dos substratos de celulose e os nanocristais de celulose resultantes. Estes resultados fornecem as primeiras dicas sobre como a variabilidade nanoestrutural da celulose isolada pode influenciar os nanocristais de celulose produzidos a partir deles.

Palavras chave: Celulose, variabilidade, nanoestrutura, nanocelulose, nanocristais de celulose, biomassa.

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Introduction

The search for renewable resources in replacement of petroleum has increased the interest and attention of research groups around the world, looking to improve the energy supply, fuel production, chemical products, and new and smart materials. This makes the research in this field multidisciplinary, allowing a multiscale and arrangement of technique and technologies for study given the possibility to resolve the obstacles the human society face. The use of vegetal biomass in the industrial production have several drawbacks, as volume of biomass needed, engineering difficulties at industrial scale for the use of biomass as raw material and, of course, the main challenge is the structural organization of biomass (macro and nanoscale). Thus, the deconstruction of biomass structure is the key factor of biorefinery research. In this context the cellulose is the main fraction of biomass to be considered to produce fuel, energy, and smart materials for large range of applications. The isolation of cellulose from different sources of biomass needs a better understanding of how the pretreatments affect its structure and how the variability of the biomass affects the nanocrystals properties. Considering the complexity of cellulose and its potential industrial applications, this study is interesting for both, academia, and industry.

This thesis is divided in four chapters that investigate the variability in nanostructure of vegetal biomass, and how this variability affects the cellulose nanocrystals production, either in terms of yield and structural characteristics.

Chapter I is a review of literature of biomass structure (macro and nanostructure), the techniques and methods to study biomass and the cellulose, the methods to extract the cellulose from biomass.

Chapter II describes materials and methods used to promote and evaluate the variability in cellulose. The chemicals treatments used to isolate and promote the nanostructure variability (hydrothermal, alkaline, and acid treatments) promote the change in nanometric structure of cellulose.

Chapter III describes results and discussions obtained from different techniques to evaluate the nanostructure variability and the correlation that appears between the parameters. The principal components analysis (PCA) supplied an easy and rapid correlation for large number of samples.

Chapter IV is the conclusions about the work, and some left perspectives left of the project.

Objectives

The general objective of this Thesis is to investigate the variability in vegetal cellulose with emphasis on nanostructural parameters and how this variability affects the production of cellulose nanomaterials, in particular, the production of cellulose nanocrystals.

The first specific objective is to understand the structural variability of celluloses isolated from plants, with emphasis on nanostructural parameters. For this goal, a variety of celluloses from different plant sources were isolated by a variety of chemical treatments. The chemical treatments can change the nanometric properties such as cellulose accessibility (porosity) and crystalline parameters, as well as water sorption behavior and chemical composition.

The second specific objective is to produce cellulose nanomaterials from a group of selected cellulose sources by using acid hydrolysis with sulfuric acid.

The third specific objective is to understand the variability in the cellulose structure and how the methods employed to produce the nanocelluloses are correlated with its properties. These correlations were made by multivariate analysis using the principal component analysis (PCA), looking for correlations between the different cellulose sources and their characteristics.

Chapter I -State of art: Literature review

1.1 - State of art: Literature review

This chapter I has the main goal to provide the necessary background of to understand these topics. As mentioned early in the introduction of this thesis, the technology advances, and the necessity of research in the field of structure of biomass and cellulosic materials.

1.2 - The need of renewable materials

Biomass can be defined as all organic material derived from living organisms (animal, vegetal, fungi and bacteria). This organic material has been used for human society since ancient times for food, clothing, or domestic utensils. During the 20th century the increase in consumption of petroleum and non-renewable sources has already showed strong negative effects on the environment (notably global warming). Having this scenario as motivation, research for new technologies to produce energy and materials from renewable sources (solar, wind, and biomass) is urgent and necessary. Here we will discuss only the vegetal biomass known as lignocellulosic biomass, processed in the context of biorefineries, because this source can provide the volume of production, availability in all places of the world, inexpensive raw materials and environmental sustainability.

Nowadays, the level of technological development provides better and efficient ways to produce and use plant biomass for energy, chemicals and materials. The integrated biomass processing facilities can be considered as biorefineries (KAMM; KAMM, 2004). The concept of biorefinery is a version of the petroleum refinery, which uses biomass as the main source of raw material. The best example of biorefinery is the typical sugarcane mill in Brazil. The sugarcane (raw material) is processed to produce food (sugar), fuel (ethanol) (GOLDEMBERG, 2008) and energy (heat and electricity) (LEAL; WALTER; SEABRA, 2013). Although the sugar industry is quite a success in Brazil for the large joint production of sugar, ethanol and electricity, there is still room for improvement because sugarcane bagasse can be a source of chemicals and new materials with higher value than current use as fuel in boilers. In recent years, several research groups have been investigating sugarcane bagasse for production of ethanol from cellulose (2G ethanol) (MACRELLI; MOGENSEN; ZACCHI,

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2012). From the point of view of the biorefinery concept, it is possible to use all types of biomass, but there are technological barriers to overcome in order to efficiently transform the biomass into a desired product (GHATAK, 2011). Furthermore, the possibility that the same process can be applied to different biomass feedstocks is still in debate. In this context the research concerning the effects of biomass structure is a key factor to help the development of biorefineries

1.2.1 -The multiscale hierarchical structure of lignocellulosic biomass

1.2.1.1 – Molecules

1.2.1.1.1 – Cellulose

Different organisms can produce cellulose, including plants (terrestrial and aquatic), fungus, bacteria, and marine animals. Cellulose is the main natural polymer and organic compound in the world. It is the most produced biopolymer in the world with production in order of 10^{12} tons per year (BERG, 2003; KLEMM et al., 2005). Due to its renewability, cellulose can be considered an inexhaustible resource. Here we will describe only cellulose from terrestrial plants because it is the main source of cellulose in the industrialized society and one of the objectives of this project is to study plant cellulose. Table I-1 shows the cellulose content for several species of higher plants (softwood, hardwood, and grasses).

Table I - 1: Cellulose content form several feedstocks for the tree main higher plant species

Feedstock	Cellulose (%)	References
Pinus	41-47	(BRITO et al., 2008)
Norway spruce	41	(EK; GELLERSTEDT;
Scots pine	40	HENRIKSSON, 2009)
<i>Eucalyptus saligna</i>	51	(BRITO et al., 2008)
Beech wood	45	(EK; GELLERSTEDT;
Brich wood	41	HENRIKSSON, 2009)
Sugarcane bagasse	40-45	(TEIXEIRA et al., 2011; SZCZARBOWSKI et al., 2014)

Cellulose samples are isolated from several plants (seeds, wood trees, flowers and others) by nitric acid and sodium hydroxide treatments.

Cellulose is the main polysaccharide in the plant cell wall, acting as the structuring element in the architecture of plant cell wall. The cellulose chain is made of β -1,4 linked anhydroglucopyranose units (AGU) (FENGEL; WEGENER, 1989; CAZACU; POPA, 2005; VARSHNEY; NAITHANI, 2011) rotated 180° from each other (HABIBI, 2014). The cellulose molecule is a linear, unbranched, polymer and is insoluble in water (ROJAS, 2016). The repeating unit of cellulose is the dimer cellobiose, but this is sometimes questionable as the point of view of the polymer nomenclature (FRENCH, 2017). The degree of polymerization (DP) of cellulose can have a wide range, from 200 to up 20,000 (Y. LIN, 1972; FENGEL; WEGENER, 1989; BERG, 2003; KLEMM et al., 2005; HABIBI, 2014), and the chain length can reach 500-15,000 nm (ROWELL; PETTERSEN; TSHABALALA, 2013; ROJAS, 2016) depending on the cellulose source. The chain ends of cellulose show different chemical groups. One end has the reducing group (aldehyde function) and the other end has a nonreducing group (glycosidic bond) (BELGACEM; GANDINI, 2008; ROJAS, 2016). Figure I-1 shows a illustration of the structural formula of cellulose.

Figure I - 1: Representation of structural formula of cellulose molecule.

Source: Author

1.2.1.1.2 - Hemicelluloses

The second class of polysaccharides in cell wall structure beside cellulose is collectively classified as hemicelluloses. Hemicelluloses comprise 10-35% of the lignocellulosic mass (EK; GELLERSTEDT; HENRIKSSON, 2009; SCHÄDEL et al., 2010) and have a more heterogeneous structure than cellulose, with different monomers units depending on plant species and cell type. Hemicelluloses are found in the cell wall matrix, in contact with cellulose microfibrils and lignin. These interactions make it quite difficult to extract the hemicelluloses without some structure modification in cellulose and lignin (SUN; HUGHES, 1998; BOBLETER, 2005; CANETTIERI et al., 2007). Wood is the main lignocellulose raw material that presents hemicelluloses, but some grasses also present these polysaccharides. The type and

quantity of hemicelluloses depend of plant species, location, growth and the extraction method (SPIRIDON; POPA, 2005; BELGACEM; GANDINI, 2008; REN; SUN, 2010).

Hemicelluloses form a group of polysaccharides that compose the cell wall matrix. Like cellulose, hemicellulose backbones are jointed by β -(1 $\rightarrow$ 4) bonds between its constituent monomers (SCHELLER; ULVSKOV, 2010; KONTTURI, 2015). The monomers found in hemicelluloses include hexoses (D-glucose, D-mannose and D-galactose), pentoses (D-xylose and L-arabinose) some deoxyhexoses (L-rhamnose and L-fucose) and uronic acids (4-O-methyl-D-glucuronic acid, D-galacturonic acid and D-glucuronic acid) (EK; GELLERSTEDT; HENRIKSSON, 2009). Table I-2 shows the main hemicelluloses content in softwood, hardwood and grasses.

Table I - 2: The main hemicellulose content in higher plants

Plant species	MGX	AGX	GlcM	GaGlcM	XylG	Glu	References
	%						
Softwood	5-15	15-30	1-5	60-70	1-15	-	(SPIRIDON; POPA, 2005)
Hardwood	80-90	~1	1-5	0.1-1	0.1	-	
Grass (P+S)	-	20-50	2-5	-	1-5	2-15	(SCHELLER; ULVSKOV, 2010)

P+S = Primary and Secondary wall; MGX= methylglucuronoxylan; AGX=Arabinoglucuronoxylan; GlcM=Glucomannans; GaGlcM=Galactoglucomannan; XylG=Xyloglucan; Glu=Glucan.

The hemicelluloses can be separated in 4 major groups that share structural similarity: (a) xylans (XGly), (b) mannans (MGly), (c) xyloglucans (XGlu) and (d) mixed-bonds of β -glucan (Mix- β -Glu) (REN; SUN, 2010). Figure I-2 shows the representation of structural formula of the main monomer's units present in hemicelluloses.

Figure I - 2: Representative structural formula of monomers presents in hemicelluloses.

Source: Author

Xylans (XGly) are the main hemicelluloses in hardwood and annual plants (cereal and crops). The amount of hemicelluloses can reach up to 30% or 50% of the lignocellulosic mass, respectively in hardwoods and in grass and cereals grains (SPIRIDON; POPA, 2005). All the xylans are made of a linear backbone of β -1-4 D-xylopyranose (xylose, Xylp) bonds with branches of arabinosyl, acetyl, and glucuronosyl groups. The methylglucuronoxylan (MGX) is the major hemicelluloses (XGly) in hardwood, the main backbone is made of xylose with side groups of O-acetyl-4-glucuronic acid (O-acetyl-4-glu-acid) at a ratio of 10:1 (Xylp:O-acetyl-4-glu-acid) (EK; GELLERSTEDT; HENRIKSSON, 2009).

Arabinoglucuronoxylan (AGX) is the major hemicellulose (XGly) in softwoods and grasses and, in some cases, also appears in lower quantity in hardwoods. The AGX hemicelluloses have a backbone of xylan with side groups of arabinofuranose (arabinose, Araf) and glucuronic acid groups. The proportion of sugar units is 1.3:2:10 (O-acetyl-4-glu-acid:Araf:Xylp) (EK; GELLERSTEDT; HENRIKSSON, 2009). In grasses, the AGX hemicelluloses contain units of ferulic acid linked to arabinofuranose units by ester linkage. The presence of ferulic acid in the hemicelluloses enables the formation of cross-linking between chains that increase the recalcitrance of biomass against enzymatic action. Arabinoxylan (AX) hemicelluloses are insoluble in water and have the same structure of AGX without glucuronic acid unit branches and can be found only in cereal grain plants. Without the side groups linked to the main chain these hemicelluloses have more compact structure due to more effective hydrogen bonds (H-bonds), which explains the insoluble nature of these polymers.

Mannans (MGly) are the main type of hemicelluloses in softwoods with the galactoglucomannans (GaGM) and glucomannans (GlcM). They are present in early plants (lycophytes and mosses) and some species of hardwoods at the secondary wall. The mannans

are made of β -(1 $\rightarrow$ 4) D-manopyranose (manose, Manp) linkages and D-glucopyranose (Glc pA) moieties. They present also some units of galactopyranose (galactose, Galp) linked as side group. Glucomannans (GluM) are the main chain made of β -(1 $\rightarrow$ 4) glycoside bonds of mannose and glucose groups. The proportion of 1:1-4 (Glc p:Manp) is distributed randomly in the chain (SPIRIDON; POPA, 2005). Galactoglucomannans (GaGM) are made of Manp and GylpA units in the main chain and side chains of Galp linked to Manp units. The proportion of galactose, glucose and mannose in galactoglucommannas is 0.1-1:1:3-4 (Galp:Glc pA:Manp) (EK; GELLERSTEDT; HENRIKSSON, 2009). The galactoglucomannas are present in softwoods, with O-acetyl group linked to mannose randomly distributed. The higher degree of galactose substitution increases the water solubility of galactoglucomannans.

Xyloglucan (XyG) is type of hemicelluloses in higher plants (angiosperms and gymnosperms). XyG represent about 2-25% of the mass in the primary wall (EBRINGEROVÁ; HRMÁDKOVÁ; HEINZE, 2005) and is made of D-glucopyranose (Glc pA) groups linked by β -(1-4) bonds with xylopyranose (Xylp) as the main side groups. (EK; GELLERSTEDT; HENRIKSSON, 2009). The XyG backbone associates to cellulose fibrils at the primary wall, cross-linking to several sites in cellulose fibrils. The interaction of side groups in XyG with cellulose was described in literature (HAYASHI; OGAWA; MITSUISHI, 1994) and is responsible to the affinity to water adsorption. This interaction is also affect by the size of XyG molecule (HAYASHI et al., 1994). This interaction gives the XyG-cellulose the ability to hold the osmotic pressure during the cell wall growth and affects positively the adsorption properties.

In summary, the hemicelluloses have the main function to surround the cellulose microfibrils in the cell wall, also making the linkage between lignin, pectin and other components in the cell wall. The hemicelluloses also participate in the adsorption properties of cell wall and the protection of cellulose. Table I-3 shows the hemicelluloses content in the plant species selected for this work, showing its natural variability in the composition. The values showed in this table cannot be compared to each other because the different methods of sugar quantification employed but can be useful for the understanding of the natural variability in these materials.

Table I - 3: Hemicelluloses monosaccharide content for different feedstocks.

Plant species	Araf	Manp	Galp	Xylp	U. A	Reference
	(%)					
Pinus	0.9	11.5	2.7	6.8	n.d	(BRITO et al., 2008)
Eucalyptus	0.4	1.5	1.3	13.1	n.d	(BRITO et al., 2008)
Sisal	2.0	1.0	1.4	15.0	1.5	(STEWART et al., 1997)
Coconut	0.8	0.1	0.3	12.6	4.2	(VAN DAM et al., 2004)
Sugarcane bagasse	1.8	n.d	0.6	20.3	n.d	(SZCZERBOWSKI et al., 2014)

Araf=Arabinose; Manp=Manose; Galp=Galactose; Xylp=Xylose; U.A= Uranic Acid; n.d=not detected

1.2.1.1.3 – Lignin

Lignin is one of the main macromolecular components in the cell wall. Lignin functions include holding together two adjacent cells, provide mechanical resistance, hydrophobicity and microbial protection for the cell wall (EK; GELLERSTEDT; HENRIKSSON, 2009). The molecular complexity of lignin is reflected in its structure, and different lignin extraction methods result in differences in lignin structure. The extracted lignin does not correspond to the lignin in the cell wall. Because of this there are still unresolved question about the structure and relationship of lignin and other macromolecules in the cell wall.

The deposition of lignin in the plant tissues occurs in the regions where the cellular division and growth is already finished. Although the biosynthesis starts with only three different precursors, the macromolecule of lignin has no repetitive units. It can be considered an oddity biopolymer because lignin is not considered a polysaccharide, lipid, protein or nucleotide (EK; GELLERSTEDT; HENRIKSSON, 2009). Lignins are random and amorphous macromolecules with a complex structure containing aromatic and aliphatic moieties, with prevalence of ether (C-O-C) and the carbon-carbon (C-C) linkages. Figure I-3 shows a structural model of lignin in softwoods.

Lignin is polymerized from monomers of phenylpropanic alcohols (monolignols), *p*-coumaryl alcohol (Hydroxyphenyl; H-type), coniferyl alcohol (Guaiacyl; G-type) and sinapyl alcohol (Syringyl; S-type). Figure I-4 show a representation of the lignin precursors.

Figure I - 3: Structural model for a softwood lignin.

Source: Adapted from EK, M., Gellerstedt, G., Henriksson, G., (2009) Wood Chemistry and Wood Biotechnology. Germany, DE Gruyter. 308 p.

Figure I - 4: Representation of the phenylpropanic units. Where R1=R2=H is p-coumaryl alcohol (H-type); R1= OCH3 and R2=H is coniferyl alcohol (G-type); R1=R2=OCH3 is sinapyl alcohol (S-type).

Source: Author

1.2.2 - Crystalline and fibrillar organization of cellulose

There are six cellulose polymorph systems known as I, II, III_I, III_{II}, IV_I and IV_{II} (O'SULLIVAN, 1997). Each polymorph is a distinct stable phase according to thermodynamic stability. Cellulose I is the polymorph found in native cellulose, i.e., in cellulose produced by living beings, Cellulose I can be converted to cellulose II either by alkali treatment (sodium hydroxide, NaOH) called mercerization (HALONEN; LARSSON; IVERSEN, 2013) or by regeneration, i.e., dissolution followed of precipitation (FU et al., 2014). Cellulose III can be formed by treatment of cellulose I or II with liquid ammonia (NH₃), and can be divided in III_I and III_{II}, where the subscript indices indicated from which cellulose polymorph it is originated (KAFLE et al., 2014). The cellulose IV is formed from heating cellulose III_I or III_{II} in glycerol, also designated as IV_I and IV_{II} respectively (WADA; HEUX; SUGIYAMA, 2004).

Cellulose I is the most studied polymorph and can assume two crystalline lattices, called cellulose I α and I β (ATALLA; VANDERHART, 1984), with cellulose I α the prevalent form in lower plants and primitive organisms (algae and bacteria) and cellulose I β the prevalent one in higher plants (wood, grass). Both types of cellulose (I α and I β) can indeed coexist in the same cellulose crystal forming cellulose I with crystallographic defects known as stacking faults. (DRIEMEIER; FRANCISCO, 2014). The crystal structures of cellulose I α and I β are described in literature (NISHIYAMA; LANGAN; CHANZY, 2002; NISHIYAMA.Y et al., 2003). The ratio of I α and I β may differ between plant species.

Cellulose II is the second cellulose polymorph more studied in academia and industry. The cellulose II can be made by two process, mercerization or solubilization. In cellulose II, the molecules present anti-parallel organization of the chains, instead of the parallel organization in cellulose I (KLEMM et al., 1998). The cellulose II is the thermodynamic stable polymorph, so the conversion from I to II is irreversible. Table I-4 shows the crystals parameters of the unit cell for the cellulose polymorphs.

The mercerization process is made by using sodium hydroxide (NaOH) solution that produced intra and interfibrillar swelling. The mercerization of cellulose occurs in a specific range of alkali concentration, from 10-25% alkali (KOLPAK; BLACKWELL, 1978; AKHBARI; ZAHIRI; BASSAM, 2012; HALONEN; LARSSON; IVERSEN, 2013; DUCHEMIN, 2015; JIN et al., 2016). The specific value of alkali concentration can be changed by temperature, time, and presence of catalysts (organic or inorganic) in the process. The alkali cellulose obtained from the alkaline treatments are interesting intermediate for processing

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cellulose, because it is more reactive (OKANOT; SARK, 1985). This is a consequence of the swollen state that has more space for solvents to interact with the hydroxyl groups of cellulose. Regeneration process is very common in industry to produce fibers of cellulose II, such as the viscose process. Dissolving cellulose is quite complex because of the amphiphilic character of the molecule and its tendency to crystallize, having crystal cores inaccessible to organic and inorganic solvents. The process to dissolve cellulose can be separated in two groups, derivatizing and non-derivatizing processes (LIEBERT, 2009). The derivatization modify cellulose by substitution of hydroxyls groups to make the cellulose more soluble. The non-derivatizing process eliminates the intermolecular bonds (H-bonds and van der Waals) and allows the cellulose to be soluble in the solvent without chemical modification of the cellulose chain (SWATLOSKI et al., 2002; MEDRONHO; LINDMAN, 2014).

Table I - 4: United cell parameters for cellulose polymorphs.

Polymorph	Crystal parameter							Ref.
	unit	a	b	c	γ	α	β	
	cell	(Å)			(deg)			
Cellulose I								
I α	T	6.71	5.96	10.4	80.37	118.1	114.0	(NISHIYAMA, et al., 2003b)
I β	M	7.78	8.20	10.38	96.50	-	-	(NISHIYAMA, et al., 2002)
Cellulose II	M	8.10	9.03	10.31	117.10	-	-	(LANGAN, et al., 2001)
Cellulose III	M	4.45	7.85	10.31	105.10	-	-	(WADA et al., 2004)
Cellulose IV	M	7.90	8.11	10.30	90.00	-	-	(KLEMM et al., 1998)

a, b, c = length axis; γ = angle between a and b axis; α = angle between b and c axis; β = angle between a and c axis; T= Triclinic; M= Monoclinic.

Microfibrils have large range of length and width depending on cellulose source. However, one aspect is common, all microfibrils are much longer than wide. The microfibrils can achieve width from 2-3 nm in the primary wall and 5-10 nm for secondary wall. The

microfibrils may have some twisted regions (BU; HIMMEL; CROWLEY, 2015; CONLEY et al., 2016). The amorphous regions occurs in a periodic distribution as show by ramie fibers studies (NISHIYAMA et al., 2003a).

Cellulose chain can make several hydrogen bonds (H-bonds), inter- and intramolecular ones, making a stable polymer, with higher stiffness in the chain direction. Along the microfibrils, there are highly ordered (crystalline regions) and less ordered (amorphous) regions (Nishiyama et al. Biomacromolecules 2003) The definition and the origin of this crystalline and amorphous regions is still not fully understood but some points are already clarified and is highly dependence of the cellulose source.

Microfibrils are made of a highly crystalline core surrounded by less ordered molecules at microfibril surfaces. This crystalline core is inaccessible even to water molecules and, therefore, the crystalline core has low reactivity.

1.2.3 - Structure of cell wall, cells, and tissues

The cell wall is the main structure of plants and has most polysaccharides present in the lignocellulosic materials. Cell wall can be classified as a composite with the cellulose fibrils as the structuring element, hemicellulose as binding element between cellulose and lignin, and lignin as a filling element with a hydrophobic character that plays an important role in liquid movement in the cells. The chemical composition of the cell wall depends on plant species, plant tissue, and growth conditions. Table I-5 shows examples of the content of the main macromolecules in cell wall for the main types of terrestrial plants: gymnosperm or coniferous (softwood), angiosperm (hardwood), dicotyledons and monocotyledons (grass and crops).

The plant tissues are made from different types of cells that can be differentiated by optical or electronic microscopy. These cells have shape, functionality and structure that fulfill the genetic function designed for them. The morphology of cells can be divided in four main classifications, parenchyma, tracheid, fibers and vessels. (GERALD, 2008).

The parenchyma cells have the main functions of i) storage, ii) metabolic pathways and iii) transportation of molecules, and it is most present in hardwoods and grasses. The tracheid have the function of long-range transport of liquids and mechanical support for the plant, and they are predominant in softwood. The fibers cells found only in hardwoods have only mechanical support function. The vessels serve exclusively to long-range transport of liquids in softwoods. They are individual cells with 50-60 cm length or longer (GERALD, 2008). The

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specific functions of these cell types affect the polysaccharide content and the ultrastructure of the cell wall.

The cell wall ultrastructure is an arrangement of cellulose, hemicelluloses, and lignin in different layers with different orientation and content of polysaccharides, defined during the cell wall construction. The arrangement and classification of these layers in the cell wall are still subject to controversies due the way the layers are defined and exhibit no clear differences between the plant species. Figure I-5 shows illustration of cell wall layers and the polysaccharides interaction.

Figure I - 5: (a) Schematic illustration of cell wall layers and the chemical composition. (b) Model of cell wall with fibrillar structure of cell wall layers for hardwood. ML=Middle lamella; P=Primary wall, S= Secondary wall (S1, S2, S3); W= Watery layer (lumen).

Source: From Gerald, K. (2008), Raw material for pulp. *In*: Handbook of pulp, H. Sixta (Ed.). DOI: 10.1002/9783527619887.ch2.

The cells are hold together by lignin in the middle lamella (ML), (EK; GELLERSTEDT; HENRIKSSON, 2009). After the ML, the next layer of cell wall is the primary wall (P), a mixture of hemicelluloses and cellulose microfibrils, having random orientation of the cellulose microfibrils. The primary wall is the thinner layer with thickness of $\sim 0.1 \mu\text{m}$ (GERALD, 2008) The next layer is the secondary layer (S), which is the stiffer and thicker layer of cell walls. The secondary wall of woods is subdivided in sub-layers (S1, S2 and S3), the quantity of sub-layers is a theme discussed in the literature because the differences between plant species. But here we will discuss the most accepted model for hardwood that presents three sub-layers in secondary wall. The differentiation of each sub-layer is determinate by the cellulose microfibrils orientation, which can vary between near perpendicular to totally parallel in relation to cell axis. The S1 and S3 layers are thinner than the S2 layer. The S2 layer represent most of the mass of the secondary layer. The last layer of cell wall is the ternary wall that is a

thinner layer between the secondary wall and the lumen (cell nucleus). The thicknesses of the cell wall layers are show in Table 5.

Table I - 5: Average thickness (μm) of softwood and hardwood. ML+P = middle lamella + primary wall; S1, S2 and S3 = secondary wall; T = tertiary wall.

Feedstock	ML +P	S1	S2	S3	T	References
	μm					
<i>Picea abies</i> (softwood)	1.00	0.26	1.66	0.10	-	(GERALD, 2008)
<i>Fugus crenata</i> (hardwood)	0.70	0.24	0.99	-	0.9	(EK; GELLERSTEDT; HENRIKSSON, 2009)

There are several models to explain the arrangement between cellulose, hemicelluloses and lignin in the cell wall ultrastructure. Each model has advantages and disadvantages and we should always be aware of misinterpretation due to the large variability between plant species and cell types.

1.3 – Cellulose accessibility

The accessibility to chemicals and enzymes is a key factor to make viable any kind of industrial process employing lignocellulosic materials as substrate. More specifically, the accessibility of lignocellulosic materials is the accessibility to cellulose because cellulose is the main component of lignocellulose used for the production of fuels and chemicals from biomass.

The porosity is a void, empty space, in the structure of biomass. The void can be inaccessible (closed) or accessible (open), connecting the inner part of the material with the exterior. The porosity of a material is defined as the ratio between the total volume of void and the volume of the solid material (LAWRENCE; JIANG, 2017).

There are several methods to measure the porosity in materials, as mercury intrusion and gas adsorption/sorption. These methods are very useful for ceramic, metallic and some polymeric materials but they are not suitable when the measurement is made in lignocellulosic substrates. The need of drying of lignocellulosic samples can produce the phenomenon called hormification (WEISE; MALONEY; PAULAPURO, 1996; FERNANDES DINIZ; GIL; CASTRO, 2004; KÖHNKE et al., 2010; DUAN et al., 2015). This phenomenon is the collapse

of the ultrastructure of the lignocellulosic substrate by reducing the pores and thus reducing the accessibility. To avoid the hornification effect, one can determine the pore size in wet state techniques such as by thermoporometry. The wet state measurement is important to verify the behavior of lignocellulosic material ultrastructure in an environment like that employed during processing treatments.

1.4 - Lignocellulose processing

To overcome the complexity in the structure of lignocellulosic materials, specific treatments (sometimes called pretreatments) are needed to promote the accessibility to lignocellulosic components and improve the conversion of the polysaccharides in its lower molecular mass components (BELGACEM; GANDINI, 2008). Several treatments are used to overcome the lignocellulosic recalcitrance (HIMMEL et al., 2007) to enzymatic or chemical processes. The treatments change the physical and chemical properties by removing the barriers of lignocellulosic components to degradation by enzymes or chemicals.

The efficiency (technical and economic) of the treatment can be accessed by applying four basic concepts; i) improve the polysaccharides accessibility without higher degradation of the target component, ii) do not produce inhibitors to enzymatic and microbiological processes, iii) use economical and available materials to convert the biomass (cheap chemical reagents and equipment) and iv) minimize the production of residues (MOSIER et al., 2005; ALVIRA et al., 2010).

1.4.1 - Hydrothermal – Liquid Hot Water (LHW) treatment

The main objective of the hydrothermal or liquid hot water (LHW) treatment is the removal of the hemicelluloses from lignocellulosic materials (GARROTE; DOMÍNGUEZ; PARAJO, 1999). The hemicelluloses removal promotes physical separation of cell wall layers, result an increase of the cell wall porosity. The LHW treatment uses water at high temperatures from 140 - 240 °C (NEGRO et al., 2003; DA CRUZ et al., 2012; HU; RAGAUSKAS, 2012) and a low concentration of dry matter 1-10% (AGBOR et al., 2011).

LHW promotes the autohydrolysis of hemicelluloses with minor chemical effects on the lignin and cellulose macromolecules. The autohydrolysis occurs by breaking the glycoside bonds in hemicelluloses thanks to the catalytic effect of hydronium ions (H_3O^+) from acetic

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acid released by the hydrolysis of hemicelluloses acetyl groups (GARROTE; DOMÍNGUEZ; PARAJO, 1999; BOBLETER, 2005). The hydrolysis of hemicelluloses produces oligomers and sugar monomers (xylose, manose, arabinose, galactose, and others) that can be dehydrated into hydroxymethylfurfural (HMF) and furfural. These degradation products are inhibitors of enzymatic and fermentation processes.

In comparison to cellulose, the hemicelluloses are easily depolymerized during LHW treatment because the lower degree of polymerization and more accessible non-crystalline structure.

One of the advantages of LHW treatment is the possibility to use lower temperatures to minimize the production of inhibitors. The treatment uses only water and does not need expensive equipment. The main disadvantage of LHW treatment is the use of diluted solutions, which produces a low concentration of dissolved sugars and oligomers in the liquor and require management of large volumes of water.

1.4.2 - Alkaline process (ALK)

The use of high pH solutions as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($\text{Ca}(\text{OH})_2$, lime) or ammonia (NH_3) in the treatment of biomass is already extensively investigated. The alkaline solution shows higher efficacy in removal of lignin and hemicelluloses with small effects on the cellulose backbone, thus improving the accessibility to enzymatic attack (CHIARAMONTI et al., 2012).

Alkaline treatments can be performed at lower temperatures and shorter times than hydrothermal treatments. There are reports in literature indicating that alkaline treatments are more effective when applied to agricultural residues (grasses) than woody materials (MOSIER et al., 2005; TAHERZADEH; KARIMI, 2008; KUMAR et al., 2009). The effectiveness of alkali treatments to the biomass fractionation are more effective in breaking linkages between lignin and hemicelluloses (ester bonds) than other methods, as acid and oxidative treatments (TAHERZADEH; KARIMI, 2008).

1.4.3 - Organosolv process

The organosolv processes employ organic solvent-water-mixtures to solubilize most of the lignin present in biomass (TAHERZADEH; KARIMI, 2008). The process is performed by heating a mixture of water and organic solvent with or without catalyst.

Organosolv process is a simultaneous action of hydrolysis and dissolution of lignin, breaking the internal lignin bonds. There is a large number of organic solvents that can be used in the organosolv processes, as methanol, ethanol, glycerol and acetic acid (CHUM et al., 1988; VÁZQUEZ et al., 1997; LIGERO; VEGA; BAO, 2005; AKGUL; KIRCI, 2009; SANNIGRAHI; MILLER; RAGAUSKAS, 2010; NOVO et al., 2011). The range of temperature used in the organosolv processes are in the range of 100-200 °C (LIGERO; VEGA; BAO, 2005; PARK et al., 2010). The solvent can be recycled by distillation and re-used in a new run, but the solid must be washed extensively with water to remove any organic solvent residue to avoid enzymatic or fermentation inhibitors.

Organosolv process performed by using organic acids can improve the delignification via dissociation of hydronium ions (H_3O^+) that catalyzes the hydrolysis and delignification of biomass (AGBOR et al., 2011).

The advantage of organosolv processes is the selectivity in lignin and hemicelluloses removal/solubilization without cellulose degradation. The lignins obtained from organosolv process are sulfur free, which means the lignin has higher purity when compared with the lignins obtained in the commercial Kraft process. The purity and the lower molecular weight enables these lignins to be used to produce phenolic compounds for polymeric materials as epoxy resins, bioplastics, polyurethane and others. Organosolv process can be combined with other treatments in a multi-stage fashion to improve the biomass fractionation for more recalcitrance biomass. Disadvantages of the organosolv processes are the cost of the solvents and, when acid catalyzed, the production of fermentation inhibitors as HMF and furfural.

1.5 - Cellulose nanomaterials

The cellulose nanomaterials are new group of materials derived from cellulose fibers that present nanometric dimension with interesting mechanical properties. They are basic divided in two types, cellulose nanofibers (CNF) and cellulose nanocrystals (CNC) (KLEMM et al., 2011). CNF and CNC differ from each other by their dimensions, the CNF present one dimension in nanometric scale, diameter 10- 100 nm with the length reaching the micron scale

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(DUFRESNE, 2012). The CNC present all dimensions in nanometric scale, diameter 1-10 nm and length 50-500 nm (ROSA et al., 2010; SIQUEIRA; DUFRESNE, 2010).

The production process to obtain the CNF and CNC is different, while the CNF is made mainly by mechanical methods as high pressure homogenizations, grinding, electrospinning and others (DUFRESNE, 2012; ROJAS, 2016), the CNC are usually made from hydrolysis of cellulose using mineral acids (sulfuric, chloric or bromic acids). The next topic will discuss more about CNC production and properties, because is the subject in study in this work.

1.5.1 Cellulose nanocrystals (CNC)

Cellulose nanocrystals (CNC) are produced by hydrolysis of the amorphous regions in high purity cellulose fibers. They are cellulose nanoparticles with high purity and crystallinity and with nanometric (~5 nm) in lateral dimensions, and shape resembling needles or rods. In literature, there are several denominations for cellulose nanocrystals, as cellulose micelles (RÅNBY, 1951), whiskers (MATHEW; THIELEMANS; DUFRESNE, 2008), cellulose whiskers (FAVIER et al., 1997; SOUZA LIMA; BORSALI, 2004; SIAUEIRA; BRAS; DUFRESNE, 2009), cellulose nanowhiskers (SÈBE et al., 2012; KHANDELWAL; WINDLE, 2013), cellulose nanocrystals (BECK-CANDANEDO; ROMAN; GRAY, 2005a) and others. In this work, we adopted the terminology of cellulose nanocrystals in agreement with TAPPI standard recommendation (TAPPI, 2011a).

To prepare CNC it is necessary to directly hydrolyze the cellulose, which is in this context cellulose previously isolated from the other macromolecules of cell walls. To obtain a pure cellulose material, as cotton, bacterial cellulose, microcrystalline cellulose (MCC), several pretreatments (mechanical, biological or chemical) should be applied before CNC production.

The common pretreatment for this purpose is an alkaline treatment using sodium hydroxide (NaOH) or potassium hydroxide (KOH) solutions followed by a bleaching process that can be made with sodium chlorite (NaClO₂) with buffer of acetate/acetic acid. The alkaline treatment is used to remove the hemicelluloses and lignin and the bleaching is aiming to remove the residual lignin. This step of pretreatment and bleaching must be made carefully, because they can promote structural modification to cellulose. The promotion of structural modification in cellulose is one of the objectives in this work, which was pursued by using different chemical treatments to isolate cellulose from several types of plant biomass.

As pointed out in the literature, CNC properties depend on the source of cellulose (DUFRESNE, 2012; IOELOVICH, 2017), but the methods of extraction can also promote some

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changes in CNC properties, as creating more hydrophilic or hydrophobic surface, superficial charge (positive or negative), or changing the crystal structure (formation of cellulose II). Table I-6 shows a group of cellulose sources and hydrolysis conditions used to produce CNC.

Table I - 6: Different cellulose sources and hydrolysis conditions for CNC production.

Source	Acid	t (min)	T (°C)	LSR (mL/g)	Reference
Kraft pulp softwood	H ₂ SO ₄ 64%	25-45	45	8.75- 17.5	(BECK-CANDANEDO; ROMAN; GRAY, 2005b)
Pinus Pinea	H ₂ SO ₄ 65%	45	45	-	(GARCIA-GARCIA et al., 2018)
Eucalyptus Kraft pulp	H ₂ SO ₄ 64%	25-45	45	8.75- 17.5	(BECK-CANDANEDO; ROMAN; GRAY, 2005b)
Acacia pulp	H ₂ SO ₄ 64%	45	45	17.5	(PU et al., 2007)
Curaua fibers	H ₂ SO ₄ /HCl (2:1)	75	45	20	(CORRÊA et al., 2010)
Sugarcane bagasse	H ₂ SO ₄ 6M	30-75	45	20	(TEIXEIRA et al., 2011)
Sisal	H ₂ SO ₄ 64%	15	60	-	(RODRIGUEZ; THIELEMANS; DUFRESNE, 2006)
Rice straw	H ₂ SO ₄ 64%	30-45	45	8.75	(LU; HSIEH, 2012)
Ramie	H ₂ SO ₄ 64%	30	55	-	(SIQUEIRA; DUFRESNE, 2010)

t=time in minutes; T= temperature in degree Celsius; LSR=Liquid solid ratio in milliliter per gram of pulp.

Since more than sixty years ago, the use of acid hydrolysis (sulfuric or hydrochloric acids) to produce suspensions of cellulose nanocrystals from different vegetal sources has been described in the literature. Rånby (RÅNBY, 1951) was one of the first to describe a production of CNC suspension. Many other strong acids were reported in literature as suitable to produce CNC's, as hydrobromic (SADEGHIFAR et al., 2011), phosphoric (CAMARERO ESPINOSA

et al., 2013), nitric and also mixtures of organic and inorganic acids (acetic and hydrochloric acid) (DAUD; LEE, 2017).

The use of sulfuric acid is the common method used to produce CNC because the obtained crystals has better ability to disperse in water, when compared with hydrochloric or organic acids. This effect occurs because the sulfuric acid promotes partial esterification of the crystal surface (ABITBOL; KLOSER; GRAY, 2013). The resulting hydrogen sulfate cellulose liberates hydronium ions (H_3O^+) and a negatively charged ($-OSO_3^-$) layer forms around the CNC, which promotes the dispersion in water. The anionic sulfate groups ($-OSO_3^-$) are randomly distributed in the CNC surface, and the total amount of ionic groups can be determined by conductimetric titration (BECK; MÉTHOT; BOUCHARD, 2015). Although the good dispersibility of charged CNC, the presence of sulfate groups is a disadvantage due to the reduction in CNC thermal stability (WANG; DING; CHENG, 2007).

The yield of CNC production is key factor to any potential application, because this parameter determined the viability in large-scale production. The time consumed in the preparation of CNC is another important issue in discussion (BONDESON; MATHEW; OKSMAN, 2006; FAN; LI, 2012; KHOSHKAVA; KAMAL, 2014). Low yields in CNC production are often reported in literature, some values are presented in table I-7.

Table I - 7: Cellulose nanocrystals yield for different cellulose sources.

Source	Yield (%)	Reference
MCC	30	(BONDESON; MATHEW; OKSMAN, 2006)
Rice straw	4.83-6.43	(LU; HSIEH, 2012)
Eucalyptus kraft pulp	10-74	(CHEN et al., 2015)
Sugarcane bagasse	50-58	(TEIXEIRA et al., 2011)
Sisal	30	(RODRIGUEZ; THIELEMANS; DUFRESNE, 2006)
Bamboo	20	(BRITO et al., 2012)

1.5.2 – Morphologic characteristics of cellulose nanocrystals

The morphologic characteristics of cellulose nanocrystals depends on the cellulose source as already explained above. Measuring this characteristic is quite a challenge because

some experimental limitations, as tip broadening in AFM (atomic force microscopy) or special resolution in SEM (scanning electron microscopy). TEM (transmission electron microscopy) is the best imaging technique to measure CNC geometry, although the difficulties in sample preparation and the required use of a contrast reagent (uranyl acetate ($\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$)). Table I-8 shows geometrical characteristics of CNC for different cellulose sources.

Table I - 8: Geometrical characteristics of cellulose nanocrystals from different sources (average values).

Source	L (nm)	D (nm)	L/D	Reference
Kraft pulp softwood	120	5	28.2	(BECK-CANDANEDO; ROMAN; GRAY, 2005b)
Kraft pulp hardwood (eucalyptus)	147	4.8	30.6	(BECK-CANDANEDO; ROMAN; GRAY, 2005b)
Microcrystalline cellulose (MCC)	105	12	9	(EICHHORN, 2011)
Sisal fiber	211	3.6	60	(RODRIGUEZ; THIELEMANS; DUFRESNE, 2006)
Sugarcane bagasse	255	4	63	(TEIXEIRA et al., 2011)
Cotton	177	12	19	(MORAIS et al., 2013)
Coconut husk	210	5.3	42	(ROSA et al., 2010)
Kenaf	330	4	82	(ZAINI; TAHIR; KARIMI, 2013)

Nanocrystals geometry parameters. L(nm) = length, D (nm) = diameter and L/D = aspect ratio.

The aspect ratio is an important parameter in CNC utilization, because this parameter determines the anisotropic phase formation and the reinforcing properties in composites. It is affected by hydrolysis condition and the aggregation of the CNC particles.

CNCs mechanical properties make CNCs attractive as reinforcement elements making the interest in the utilization of CNC in composites an intense area of research (OKSMAN et al., 2006; DUFRESNE; BELGACEM, 2010; LENG, 2016). CNC particles have outstanding mechanical properties, as high Young's Modulus up to 150 GPa (FAVIER et al., 1995) low density ($\rho \approx 1.62 \text{ g/cm}^3$), high aspect ratio (L/D) (see table I-8).

Chapter II - Materials and Methods

2.1 - Biomass feedstocks

We selected six vegetal sources for cellulose isolation processes. The raw biomass feedstocks were sisal fiber, coconut fiber, sugarcane rind, sugarcane pith, eucalyptus sawdust and pine sawdust. The sisal fiber was purchased from SisalSul Fibras Naturais Ltda., São Paulo, Brazil. The coconut husk was kindly given by EMBRAPA Agroindustrial Tropical, Ceara, Brazil. The sugarcane plants were kindly given by Usina Ipiranga de Açúcar e Álcool Ltda., São Paulo, Brazil. The eucalyptus and pine sawdust were given by the Laboratório de Madeiras e Estruturas de Madeiras (LaMEM) from Universidade de São Paulo (USP), Campus São Carlos, São Paulo, Brazil. In addition to these raw biomass sources, the following commercial celluloses were used as reference materials: microcrystalline cellulose Avicel PH-101, eucalyptus dissolving pulp and bleached eucalyptus kraft pulp. The Avicel PH-101 was purchased from Sigma Aldrich. The eucalyptus dissolving pulp was kindly given by Bahia Pulp Ltda, Camaçari, Bahia, Brazil. The bleached eucalyptus kraft pulp was given by Cenibra S.A., Belo Horizonte, Brazil. For simplification for this point forward we will use abbreviation in the name of the materials as described in table II-1.

Table II - 1: Abbreviations of the materials names used during this work

Lignocellulosic material	Abbreviation
Coconut fiber	CCF
Cotton linter	CTL
Eucalyptus sawdust	ESD
Pine sawdust	PSD
Sisal Fiber	SIF
Sugarcane rind	SCR
Sugarcane pith	SCP
Dissolving pulp	DIS
Eucalyptus Kraft pulp	EKP
Avicel	MCC

2.1.1 – Sugarcane rind and pith fractions (SCR and SCP)

The sugarcane is a perennial grass crop extensively used as food, fuel and energy source mainly in developing countries of South America, Asia, African and the Caribbean. The sugarcane plant can be divided in roots, stalk and leaves, with differences in chemical composition and morphologies (BAKKER, 1926). The stalk is the most used fraction in the sugarcane industry and is composed by nodes and internodes. The nodes are the attachment points of the leaves and the internodes are the segments between the nodes. The length of internodes changes from the bottom to the top of the plant and is affected by the plantation environment. The internode can be separated in two regions, peripheral (rind) and inner (pith) (VAN DILLEWIJN, 1952). The rind and pith region differ mainly by sugar-juice content that is higher in pith (rich in parenchyma cells) than rind (rich in fibrovascular bundles). The rind is recovered by wax that protects the plant.

The Brazilian sugarcane production in the harvest 2016/2017 was over 657 million tonnes (CONAB, 2017), concentrated in the Center-South region. This means a huge amount of raw material supplying the sugarcane industry.

For this project the sugarcane rind and pith fraction were obtained by fractionation of the sugarcane (HOI; MARTINCIGH, 2013) as the schematically shown in figure II-1. First, the leaves of sugarcane were removed from the stalks, which were cut in small segments near the nodes to separate the internodes. The internodes were drilled with a bench drill separating the periphery (rind) from the inner mass (pith). The rind was extracted in a soxhlet system with n-hexane for 24 hours to remove wax and extractives, followed by extraction with distilled water for more 24 hours. The pith fraction was pressed with a hydraulic press with 10 ton of load ($\sim 4,4 \times 10^6$ Kg/m²) for 5 minutes to remove the sugarcane juice, which was followed by exhaustive washing with tap water to remove all sugar juice residues. After washing the rind and pith fractions were dried at room temperature up to 10% moisture content.

Figure II - 1: Schematic representation of sugarcane fractionation. a) Fractionation of the sugarcane plant in rind and pith fractions. b) Removal of extractives and residual sugars.

Source: Author

2.1.1 - Sisal fiber (SIF)

Sisal is an agave-type plant that grows during all year and can be cultivated in semiarid soils, thus being an option for cultivation in poor soils (CRUZ; DIERIG, 1996). The sisal fiber used in textiles, food (alcoholic and nonalcoholic beverages), craftwork and construction. Brazil is the largest producer of sisal fiber in the world with 180 tons in 2016 (FAO, 2018a) and the northeast region is the main producer, mainly from small-scale family farming. The sisal fiber obtained from the sisal leaf by decortication. The remaining solid (about 5% of initial mass) is

the fiber itself and the other 95% is a liquid residue that can be used as animal feed and fertilizer (LIMA et al., 2014).

The sisal macromolecular composition is about 43% cellulose, 32% hemicelluloses and 15% lignin (CRUZ; DIERIG, 1996). The use of sisal fibers for nanocomposites (RODRIGUEZ; THIELEMANS; DUFRESNE, 2006) based on cellulose nanocrystals and nanofibrils is new trend in research groups. Figure II-2 shows pictures of the sisal plant and the schematic of fiber production.

Figure II - 2: In a- Photography of sisal plant b- Schematic of sisal fiber production and c- Photography of sisal fiber.

Source: a and b - Wikipedia. c – author.

2.1.2 - Coconut fiber (CCF)

Coconut is produced for the food industry where coconut is used for food and non-food production. The green coconut is consumed as coconut water, while the mature coconut is the source of white meat. Coconut shells are a troublesome residue for cities due to difficulties for proper disposal and management of this residue, which usually goes to landfills. The green coconut is composed by 65% of the nut (albumin and water) (ROSA et al., 2002) and about 35% is the coir (mesocarp tissue) (TAN; AHMAD; HAMEED, 2008). The processed coir is composed by two fractions. The first is the long fibers used to produce carpets and ropes at artesian manufactures. The second fraction is a mixture of short fibers and thin powder used as soil fertilizer. The world production of coconut in 2016 was over 59 million tons and the Brazilian production in this year was 2.6 million tons and the coir production was 100,000 tons (FAO, 2018b).

The chemical composition of coir is about 38-40% lignin, 43-53% cellulose, and 15% hemicelluloses (PEREIRA et al., 2015). New ways for using the coir in new products and materials are an active research topic in several groups in academia and industry. In this context, the production of composites using coir as reinforcement agent is common (JOHN; THOMAS, 2008) as well as the production of nanocelluloses (NASCIMENTO et al., 2014). The figure II-3 present open coconut shell with the identification of coconut parts.

Figure II - 3: Green coconut. In a - Indication of coconut parts, b- Coir.

Source: Adaptation from FAO (Food and Agriculture Organization United Nation)

2.1.3 - Cotton linter (CTL)

Cotton is a renewable fiber used in human society since the ancient Egyptians when cotton was used in textile production. Nowadays, cotton has several applications in paper and textile industries, chemical production (cellulose derivatives) and composite materials. Beyond wood, cotton is a common source of cellulose, because of its high cellulose purity and low contents of lignin and hemicelluloses, requiring milder processes to isolate cellulose, and generate soft (CRUZ; DIERIG, 1996). Cotton processing generates cotton linter as byproduct. Cotton linter is classified in three types: first and second cut (depending on the number of applied delinting steps) and millrun (byproduct of the oil production from the cotton seeds) (SCZOSTAK, 2009).

The world production of cotton linter was over 26 million tons and the biggest producer is India with more than 6 million tons in 2014. Brazil was the fifth producer with 1.4 million tons (FAO, 2018c). Figure II-4 shows the cotton flower and the cotton around the seeds.

Figure II - 4: Representations of the cotton flower and location of cotton linter

Source: Author

2.1.4 - Eucalyptus and Pine sawdust (ESD and PSD)

Sawdust (also wood dust) is a residue of woodworking (grinding, sawing, planing, drilling and sanding). Sawdust is a major issue in wood industry because of its high flammability and health hazard associated with particle inhalation. Nevertheless, sawdust can be used as fuel, absorbing oil, heating isolation, cat litter and others.

In 2016 the world production of wood residues (sawdust and chips) was over 236 million m³ (FAO, 2018d). This amount of raw material can be used as fuel in furnaces to produce heat or electricity, for industry and houses. The house heat isolation is very common application for sawdust, because is a cheap material and easy replaceable

2.1.5 - Dissolving pulp (DIS)

Dissolving pulp (DIP), also known as dissolving cellulose, is a bleached cellulose pulp with cellulose content greater than 90%. DIP is made from bleached wood pulp or from cotton linter, has high brightness, low hemicelluloses content and lower polydispersity. DIP is cellulose not made for paper, but for other applications requiring fiber dissolution (viscose production) or chemical derivatization (acetylation, etherification, nitration and other).

The process of producing DIP from wood pulp requires an acid-hydrolysis step performed before the Kraft pulping process. This additional step aims at removing the hemicelluloses which would otherwise remain in the pulp. The pulp labelled as having 98% of α -cellulose content. The world production of dissolving pulp in 2016 was 6,3 million tonnes,

43% produced in the Americas (2,7 millions tonnes), with Brazil corresponding to 10% of world production and the only producer in South America (FAO, 2018e).

2.1.6 - Eucalyptus Kraft pulps (EKP)

The Kraft process is a pulping process developed at the end of 19th century by Carl F. Dahl (DAHL, 1884). The Kraft process gained popularity and economic viability in 1930s by the introduction of the alkali recycling step with the invention of the recovery boiler by G.H Tomlison (SIXTA, 2006). Since then, Kraft pulping became the main process in the pulp and paper industry to produce higher quality cellulose fiber.

The Kraft process works by cooking wood chips (hardwood or softwood) in alkaline system with sodium hydroxide (NaOH) and sodium sulfide (Na₂S). Wood components lignin and hemicelluloses are dissolved in the process, forming the black liquor rich in lignin, hemicelluloses and the used chemicals (NaOH and Na₂S). The cellulose fibers are separated from the other components by washing, filtration and centrifugation. The unbleached pulps then go to a bleaching process to achieve the require brightness (TRAN; VAKKILAINNEN, 2012). Figure II-5 shows a schematic representation of the Kraft pulping in the industry.

Figure II - 5: Schematic Kraft pulping process, with focus in the recovery.

Source: Adapted from; Tran, H., Vakkilainen, E. K., The Kraft chemical recovery process. TAPPI Kraft Recovery Course. 2012, p. 1-8.

The global chemical pulp production in 2016 was more than 139 million tons and this 137 million is Kraft pulp (bleached and unbleached), with Brazil corresponding to 13% of this production (FAO, 2018e). In Brazil, Kraft pulp is produced mainly from eucalyptus because of

its fast-growing rate, high yield per hectare, weather adaptability (grow in any region of Brazil), and large range of wood application (paper, furniture, and fuel)

2.1.7 - Microcrystalline cellulose (MCC)

Microcrystalline cellulose (MCC) is a cellulose material partially depolymerized by acid hydrolysis with mineral acids (chloric and sulfuric). The MCC is use as filler and binder agent in tableting formation in the pharmaceuticals and food industry. The common source for MCC production is cotton and wood pulp, but other cellulose source can be used for MCC production, such as sisal (BHIMTE; TAYADE, 2007), sugarcane bagasse (PADMADISASTRA; GONDA, 1989), oil palm (MOHAMAD HAAFIZ et al., 2013) and agricultural residues (ELSAKHAWY et al., 2007).

2.2 - Cellulose isolation

Figure II - 6: Schematic workflow of cellulose isolation and nanostructure characterization

Source: Author

The figure II-7 show the workflow of cellulose isolation process. The condition used to perform each process is show.

Figure II - 7: Workflow for cellulose isolation from biomass

Source: Author.

LHW = Liquid Hot Water (hydrothermal), ALK = Alkaline (soda), ACT = Acetosolv (acetic acid).

The six raw biomasses (CCF, ESD, PSD, SIF, SCR and SCP) The raw biomasses submitted to four chemical treatments: hydrothermal (LHW, liquid hot water), alkaline (ALK, soda), hydrothermal followed by alkaline (LHW-ALK) and acetosolv (ACT, acetic acid).

The LHW treatment was perform in a batch reactor of 195 mL stainless steel (304 grade) with a polytetrafluorethylene (PTFE) O-ring, heated in a thermostatic glycerol bath. The treatments were made with solid-liquid ratio of 1:10 (mass:volume) at 180 °C for 1 hour. The system is cooled in an ice-water bath, the reactor content was filtrated in and washed until a neutral pH. The solid was dry at room temperature until 10% moisture content. The condition (time and temperature) were selected based on a previous group study showing this condition promotes extensive hemicellulose removal with minimum cellulose degradation (OLIVEIRA, 2014).

The ALK treatment was perform in a batch reactor of 195 stainless steel (304 grade) with a sealing polytetrafluorethylene (PTFE) O-ring. The reactor was heat in a thermostatic glycerol bath. The treatment is made with a solid-liquid ratio 1:10 (dry mass: volume) at 160 °C for 1 hour. The alkaline solution is made of 2% of sodium hydroxide (NaOH) and 0.15% wt. of anthraquinone as catalyst for the pulping. The system is cooled in ice-water bath, the reactor content was defibrillated with alkaline solution of 1% NaOH for 5 minutes. After the defibrillation, the solid was filtrated and washed with distillated water until a neutral pH. The

solid was dry at room temperature until 10% moisture content. The LHW-ALK treatment was LHW treatment followed to ALK following the same methods describe above.

The ACT treatment was performed in a glass reactor of 1 L in a thermostatic glycerol bath under reflux. The treatment used a solid-liquid ratio 1:20 (dry mass:volume) at 110 °C for 3 hours. The solution used has 93% of acetic acid (CH_3COOH , 17.4 M), 0,3 mL of hydrochloric acid (HCl , 12.2 M) and 6.7% of distillated water. At the end of the reaction the solid was wash with hot acetic acid (at 80°C) to remove the lignin condensation over the biomass. Then the solid was wash with distillated water until neutral pH. The solid was dry at room temperature until 10% moisture content.

2.2.1 - Determination of dry mass in reaction (input and output)

The moisture content and the dry mass of the materials were measured by a thermobalance TopRay series from Bel Engineering. We used 0.5 g of biomass in the thermobalance were used period 10-30 minutes (longer time for higher moisture), the result was given by the thermobalance as percentage of moisture in the sample (w). Equation 1 calculates the dry mass content m_d as function of the wet mass m_b and the sample humidity.

$$m_b(g) = m_b \times \left(\frac{100-w}{100}\right) \quad (1)$$

2.2.2 - Bleaching process

The bleaching process used the chlorite method to remove lignin. The process was performed with initial suspension of 3% in biomass (40 g of biomass in 1280 mL of water) 14.4 g of sodium chlorite (NaClO_2) and 4 mL of acetic acid (CH_3COOH , 17.4 M) were mixture to the suspension. The suspension was mechanically stirred and heated to 70°C for. At the end of the first and second hour, 0,5 mL of acetic and 1,5 g of sodium chlorite. At the end of the three hours period, the suspension was washed with methanol and cold water until the chlorite smell and color (solid has yellow-green color) was removed and the solution reached neutral pH. The solid was dried at room temperature until 10% moisture content.

2.3 - Characterization of cellulose substrates

2.3.1 - Chemical composition

Chemical composition analysis based on the Klason method was used to quantify the contents of lignin, hemicelluloses and cellulose. Lower lignin contents in bleached pulps needs the use of Kappa number determination.

The Klason method was performed as described by Novo (2012), which is a modification of the method from TAPPI report “Acid-insoluble lignin in wood and pulp” (TAPPI, 2011b). The modified method uses a two-steps hydrolysis of biomass. The first step was performed at room temperature with 0.8 g of grinded biomass (particles smaller than 0.42 mm), which was hydrolyzed with 12 mL of sulfuric acid at 72% (w/w) for two hours under magnetic agitation. For the second step the suspension is diluted with distilled water up to an acid concentration of 3% (addition of 450 mL of distilled water) and autoclaved at 120 °C for 1 hour. After the autoclave post-hydrolysis, the suspension was filtered in a weighted sintered funnel ASTM 10-15. The solid is the insoluble lignin and the solution contains soluble lignin, sugars and sugar degradation products.

Insoluble lignin is quantified gravimetrically, the sintered funnel (from last step) is dry at 110°C in oven for 12h. The solid dry mass is the insoluble lignin. The lignin mass is corrected for inorganic content (ash). The ash is determinate by measure the residual mass after burning the lignin at 525°C for 4 hours. The ash content is used to correct the lignin content, which is expressed in percentage of initial dry mass as shown in Equation 3.

$$(L_{ins}(\%)) = \left(\frac{m_{ins} - m_{ash}}{m_{biomass}} \right) \quad (3)$$

In Equation 3, L_{ins} is the percentage of insoluble lignin in the biomass dry mass, m_{ins} is the difference of the sintered funnel mass (with solid minus empty), $m_{biomass}$ is the dry mass of biomass (~0.8 g).

Soluble lignin was quantified by UV-VIS spectroscopy as described by Goldschmidt (1971), following Equation 4. The UV-VIS spectra were obtained in the range of 190–320 nm, using a 10 mm polish quartz cell with a spectrophotometer DR-5000 from Hach.

$$L_{sol}(\%) = \left\{ \frac{[(4.53 \times A_{215}) - A_{280}] \times V_f \times F_d}{3 \times m_{biomass}} \right\} \quad (4)$$

In equation 4, L_{sol} is the percentage of soluble lignin in the dry biomass, A_{215} and A_{280} are absorbances at wavelengths 215 and 280 nm, respectively, V_f is the final volume of the hydrolysate solution after filtration, F_d is the dilution factor used to obey the Beer law in the absorbance spectra and $m_{biomass}$ is the mass of the dry biomass. The total Klason lignin (KL) in the sample is the sum of L_{ins} and L_{sol} as shown in equation 5.

$$KL (\%) = L_{ins}(\%) + L_{sol}(\%) \quad (5)$$

Monosaccharides content in the hydrolysates were quantified by high-performance liquid chromatography (HPLC), using a Shimadzu chromatograph with refractivity index and UV detectors.

Quantification of monosaccharides and organic acids (glucose, xylose, arabinose, mannose, formic acid, acetic acid, and levulinic acid) were performed using Aminex HPX-87H (Bio-Rad) column at 45°C and 5 mM of sulfuric acid as eluent with flux 0.6 mL/min with refractivity index detector RID-6A from Shimadzu. The degradation products (hydroxymethylfurfural and furfural) were detected with u-Bondapack C18 Waters column at room temperature (30 °C) with mixture 60/40 of the water/acetonitrile and acetic acid as eluent at 0.8 mL/min using UV-detector at wavelength 274 nm.

The conversion of the concentration of monosaccharides and degradation products in cellulose and hemicelluloses followed the inverse degradation route of cellulose and hemicellulose in acidic medium (GURGEL, 2010).

2.3.2 - Ash content in raw materials

The ash content in raw materials was measured according to TAPPI 211 om-02 (TAPPI, 2002). About 1 g of dry raw material was placed in a porcelain crucible with knowing mass. The crucible was placed in a muffle furnace at 525°C for 4 hours. After the dry oxidation, the crucible was cooled to room temperature inside a desiccator under low pressure for 1 day. The crucible was weighed, and the ash content was determined as the mass percentage of the initial raw material.

2.3.3 - Quantification of lignin residues

Lignin contents in the bleached pulps were determined by the micro-Kappa number standard tests (TAPPI, 1991). The micro-Kappa number is the same test of the Kappa number, with the difference that the micro version is used to quantify the lignin content in fully bleached pulps and materials. The Kappa number measures the amount of potassium permanganate (KMnO_4) that reacts with the pulp in each time.

The micro-kappa test was performed using 1 g of bleached pulp disintegrated in 80 mL water. After complete disintegration, 20 mL of a solution of 0.1 mol/L of KMnO_4 and 2 mol/L of H_2SO_4 (with 10 mL of each reagent) was added to the pulp and mixed for 10 minutes. After 10 minutes, the solution is titrated with 0.01 mol/L of sodium thiosulfate (Na_2SO_3) using sodium iodine (KI) as indicator. All the experiments were made in duplicate and the deviation is reported as the standard deviation.

The kappa number (K) calculation is show in equations 6.a and 6.b, which are identical to the equations described at the standard test TAPPI kappa.

$$K = \frac{(p \times f)}{w} \quad (6.a)$$

$$p = \frac{(b-a)}{0.1} \quad (6.b)$$

In the Equation 6.a, p is the amount in milliliters of KMnO_4 consumed during the test, f is the correction factor of consumed KMnO_4 , w is the dry mass of the pulp used in the test. In Equation 6.b, b is the amount in milliliters of Na_2SO_3 consumed in the blank test and a is the amount in milliliters of Na_2SO_3 consumed in the pulp test.

2.3.4 - Thermoporometry (TP-DSC)

Themoporometry followed the method developed and used at CTBE/CNPEN (DRIEMEIER; MENDES; OLIVEIRA, 2012; MENDES; LING; DRIEMEIER, 2016).

The air-dried samples (cellulose pulps) were soaked in deionized water for 24 hours to remove any soluble component. Then, 1-3 mg of sample is placed in aluminum pan (Tzero®). Deionized water is added in excess to the sample to guarantee the sample is saturated with water.

Measurements were performed in a DSC Q200 calorimeter (TA Instruments) coupled to a RCS90 cooling unit and an autosampler. The sealed pan is placed in the calorimeter, where

the sample is initially cooled to -70°C followed by a stabilization isotherm that guarantees water freezing. The next steps were controlled heating steps up to 5°C . Each step is composed by a heating ramp of $1^{\circ}\text{C}/\text{min}$ followed by an isotherm to equilibrate the base line. The temperatures of isotherms were: $-70, -60, -50, -40, -30, -20, -15, -10, -6, -4, -2, -1.5, -1.1, -0.8, -0.5, -0.2$ and 5°C . The temperature depression ΔT of ice melting is related to pore diameter (d) as describe in Gibbs-Thompson equation, $d = 2K_c/\Delta T$, where $K_c = 19.8 \text{ nm K}$.

The Heat (Q_i) of ice melting for the different heating steps ($i = 1, \dots, N$) was calculated by time integration of the heat flow, correct by baseline as the analytical method. The thermoporometry results, pore size distribution gives by a cumulative value of freezing bound water (FBW), in units of grams of water per grams of dry mass, as a function of pore diameter. The amount of non-freezing bound water (NFBW) is estimated by the difference between total water and freezing bound water. Figure II-8 shows the heat program and the measured heat flow during a measurement. Figure II-9 shows a profile of the pore size distribution.

Figure II - 8: Thermoporometry program. Temperature as a function of time (red), measured heat flow in the sample as a function of time (blue).

Source: Author, experimental data obtained for this project

Figure II - 9: Pore size distribution for a sample of raw sugarcane rind.

Source: Author, data from laboratory experiments for this project

2.3.5 - X-ray diffraction (XRD)

X-ray diffraction (XRD) followed the procedure developed and used in CTBE/CNPQM (Oliveira and Driemeier 2013). Samples for XRD were placed inside special glass capillary tubes with 2 mm diameters and 0.01 mm wall thickness (Chales Supper). The tubes were positioned perpendicular to the X-ray beam provided by an ultraX-18HF rotating anode generator (Rigaku) with CuK α radiation ($\lambda = 1.54 \text{ \AA}$) monochromator by VariMax HR optics. The scatter pattern was acquired by a mar345 image plate from Marresearch positioned 160 mm behind the samples. The calibration of the position and tilt of image plate were made with α -alumina as describe by Driemeier and Calligaris (DRIEMEIER; CALLIGARIS, 2011). Figure II-10 shows the geometry used for the XRD measurements.

Figure II - 10: Experimental setup for two-dimension XRD, with the representation of the principal angles that describe crystallite orientation.

Source: Adaptation from Oliveira, R. P, Driemeier, C. CRAFS: A model to analyze two-dimension X-ray diffraction patterns of plant cellulose. Journal Applied of Crystallography. 2013. 46, 4, p. 1196-1210. DOI: 10.1107/S0021889813014805.

XRD patterns were analyzed using the CRAFS model (OLIVEIRA; DRIEMEIER, 2013), which fits the 2D XRD pattern using a tailor-made Rietveld refinement model and computer code. The CRAFS model is executable in a MATLAB programming. Figure II-11 shows the CRAFS data analysis.

Figure II - 11: Example of CRAFS data analysis. In a- Two-dimensional diffraction patterns, for three samples. Experimental (top), calculated (middle) and residue (bottom) are presented. In b- Diffraction pattern for filter paper at 90° (top) and 0° (bottom) with experimental data (grey), calculated data (red), background (black). Selected peaks are indexed.

Source: Adaptation from Oliveira, R., Driemeier, C., CRAFS: A model analyze two-dimensional X-ray patterns of plant cellulose. Journal of Applied Crystallography. 2013. 46, (4), p. 1196-1210.

2.3.6 - Dynamic vapor sorption (DVS)

DVS was performed in a vapor sorption analyzer Q5000-SA (TA Instruments) following the method used in CTBE/CNPEM. (DRIEMEIER; MENDES; OLIVEIRA, 2012). In the DVS measurements, the relative humidity (RH) in the sample environment is controlled and the changes in sample mass are measured. The RH control is performed through modulation of the N₂ (RH=0) and water vapor (RH=1) flows through the sample chamber. DVS measurements were made at 50°C, starting with samples with ~10% moisture content submitted to an initial environment at RH=0.95. Then, RH is decreased stepwise to RH=0.9 and further decreased in steps of 0.1 up to complete dryness (RH = 0). Then, the steps are reversed until RH reaches 0.95 again. Each RH step takes 60 minutes, except RH=0 (dry), which is extended to 180 minutes to insure complete water removal from the sample, which provides the reference of sample dry mass. Figure II-12 shows the desorption and sorption raw data used for analysis.

Figure II - 12: Raw data of Avicel PH-101 from a DVS measurement, showing the desorption and sorption branches.

Source: Author, data from laboratory measurements of a sample used in this project

Data analysis followed the CTBE/CNPEM method (DRIEMEIER; MENDES; OLIVEIRA, 2012). The moisture desorption and sorption isotherms (Figure II-13) were constructed based on masses measured at the end of each RH step. Isotherms were fitted by the Hailwood-Horrobin sorption model (HH-model) (HAILWOOD; HORROBIN, 1946). Figure II-13 shows examples of the sorption and desorption curves fitted with the HH model.

Figure II - 13: Desorption (solid dots) and sorption (empty dots) isotherms from Avicel and S-101celluloses. Fits form Hailwood-Horrobin model (lines).

Source: Adaptation form Driemeier, C., Mendes, F. M., Oliveira, M.M., Dynamic vapor sorption and thermoporometry o probe water in celluloses. *Cellulose*. 2012. 19, (4), p. 1051-1063.

2.3.7 - Infrared spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) was performed in the attenuated total reflection (ATR) mode using the Spectrum 400 spectrophotometer (Perkin Elmer) with universal ATR accessory with diamond-covered ZnSe crystal.

The analyzed samples were powders of cellulose pulps. Spectra were acquired in 650 – 4000 cm^{-1} range with 4 cm^{-1} resolution and averaging of 16 scans. Flat baseline was subtracted from the raw spectra and the base-line corrected spectra were normalized by the highest peak intensity at 1035 cm^{-1} , which is in the carbohydrate spectral fingerprint region.

2.4 – Nanocellulose

2.4.1 - Production of cellulose nanocrystals (CNC)

Cellulose nanocrystals (CNC) were prepared by acid hydrolysis with 64% wt. sulfuric acid (H_2SO_4) at ratio solid-liquid of 1:8.75. This ratio was used in several previous works (DONG; REVOL; GRAY, 1998; BECK-CANDANEDO; ROMAN; GRAY, 2005b; JONOBI et al., 2015). The system was heated at 45°C for 30 minutes under mechanical agitation. The reaction was stopped by adding ice cubes of Milli-Q water and diluted 10-fold. The resulting suspension of cellulose was centrifuged 5 times at 4400 rpm for 10 minutes for removal of the excess of acid and sugars formed during hydrolysis. In the first centrifugation the supernatant

was separated for estimation of sugar concentration using chromatographic analysis. For the other four centrifugations the supernatants were discarded because of the lower sugar and higher acid contents. Following the centrifugation, the remaining cellulose gel was dialyzed against tap water until neutral pH. The suspension was sonicated with an ultrasonic probe in an ice bath for 5 minutes. Then the suspension was filtered in a Nylon filter membrane of 1 μm porosity (Whatman). Then the solution was dried by lyophilization, to obtain a powder of CNC. Figure II-14 shows a schematic process for CNC production.

Figure II - 14: Schematic diagram of CNC production from cellulose pulp.

Source: Author

2.4.2 - Characterization of cellulose nanocrystals

The cellulose nanocrystals were characterized the yield in cellulose nanocrystals production measure gravimetrically, the monosaccharides production by liquid chromatography measurements and remained un-hydrolysate solid by gravimetrically measure. The morphology of cellulose nanocrystals was measured by atomic force microscopy (AFM). The CNC cellulose crystal structure was analyzed by X-ray diffraction (XRD).

2.4.2.1 - Yield in cellulose nanocrystals production

Cellulose nanocrystals (CNC) production three parameters are measured, CNC yield, sugar production and unhydrolyzed solid. The mass balance of CNC production was made by measured three products of cellulose hydrolysis. First, dried mass of CNC (obtained after lyophilizing) is weighted. Second, solid non-hydrolysate (solid reaming in the filtration membrane) is weighted. Third, monosaccharides content of cellulose molecule hydrolysis during the acid hydrolysis. The CNC yield, monosaccharides content and non-hydrolysate solid is given as percentage of the initial cellulose pulp mass, as described in the equation 7.

$$R (\%) = \frac{m_n}{m_{pulp}} \times 100 \quad (7)$$

In equation 7, R (%) is the parameter measure (R = CNC yield, monosaccharide content or non-hydrolysate solid), m_n is the mass of the interesting component (CNC, sugar or solid) and m_{pulp} is the start mass of cellulose pulp used in the hydrolysis. The sum of the three parameters, CNC yield, sugar content and non-hydrolysate solid, the mass balance of nanocellulose production

2.4.2.2 – Monosaccharides quantification

Monosaccharides content in the hydrolysates were quantified by high-performance liquid chromatography (HPLC), using a Shimadzu chromatograph with refractivity index and UV detectors.

Quantification of monosaccharides and organic acids (glucose, xylose, arabinose, mannose, formic acid, acetic acid, and levulinic acid) were performed using Aminex HPX-87H (Bio-Rad) column at 45°C and 5 mM of sulfuric acid as eluent with flux 0.6 mL/min with refractivity index detector RID-6A from Shimadzu. The degradation products (hydroxymethylfurfural and furfural) were detected with u-Bondapack C18 Waters column at room temperature (30 °C) with mixture 60/40 of the water/acetonitrile and acetic acid as eluent at 0.8 mL/min using UV-detector at wavelength 274 nm.

2.4.2.3 - Nanocellulose morphological characterization

The morphology of CNC was measured by atomic force microscopy (AFM). The measures were performed using NanoSurf Flex atomic force microscope at Department of Materials Engineering from University of São Paulo, campus São Carlos. A few drops of the dilute suspension of CNC (0.001% wt) were deposited in freshly cleaved mica substrate, then allow to dry. AFM images were analyzed by the free software Gwyddion, for the measurement of length and width of CNC's

2.4.2.4 - Crystalline characterization of cellulose nanocrystals

X-ray diffraction was done following the same procedure described earlier in this chapter (subsection X-ray diffraction). The CNC samples were prepared for XRD, making films in TEFLON plates by casting. The films were transformed into powders placed inside special glass capillary tubes with 2 mm diameters and 0.01 mm wall thickness (Chales Supper).

Chapter III - Results and discussion

3.1 - Variability of lignocellulosic feedstocks

3.1.1 – Chemical composition

Chemical compositional analysis was the first characterizations made in the raw and commercial materials. This characterization aims at revealing the preexisting variability in the selected materials. The chemical composition of raw lignocellulosic materials is showed in table III-1. The deviation showed in the table is the standard deviation of duplicate analyses

Table III - 1: Chemical composition of raw lignocellulosic feedstocks and commercial celluloses.

Feedstock	Cellulose	Hemicelluloses	Lignin	Ash	Total
	%				
Coconut fiber	36.3 ± 0.2	21.5 ± 0.2	33.3 ± 0.9	2.8 ± 0.9	93.9 ± 0.9
Cotton linter	94.6 ± 0.2	n.d	2.7 ± 0.5	1.7 ± 0.8	98.9 ± 0.8
Sawdust					
Eucalyptus	47.7 ± 0.5	14.6 ± 0.5	28.8 ± 0.8	n.d	101.2 ± 0.7
Pine	55.1 ± 0.2	14.5 ± 0.5	30.4 ± 0.8	n.d	100.1 ± 0.8
Sisal fiber	60.1 ± 1.1	19.4 ± 1.4	13.3 ± 0.9	3.2 ± 0.9	95.9 ± 1.1
Sugarcane					
Rind	48.0 ± 0.5	26.9 ± 0.5	25.4 ± 0.9	0.7 ± 0.2	100.9 ± 0.9
Pith	47.7 ± 0.8	31.3 ± 0.5	23.8 ± 0.8	0.9 ± 0.1	102.8 ± 0.8
Avicel	98.5 ± 0.6	2.4 ± 0.9	n.d	n.d	100.9 ± 0.9
Eucalyptus Kraft pulp	74.8 ± 0.5	17.3 ± 0.7	n.d	n.d	92.1 ± 0.7
Dissolving pulp	100.8 ± 0.8	0.2 ± 0.1	n.d	n.d	101 ± 0.9

Total = cellulose + hemicellulose + lignin + ash; n.d = not detected.

For the chemical compositions showed in table III-1, one important result is the total over 90% for all samples, showing a good accuracy in the compositional analysis. Our compositional data (Table III-1) are like earlier reports in literature that were presented in chapter I.

3.1.2 – Cellulose crystal parameters

The cellulose I crystals parameters measured by XRD are presented in figure III-3. The unit-cell parameters (a , b , c and γ), the calculated interplanar spacing (d_{200}), and crystallite width (L_{200}) are presented.

Figure III - 1: Cellulose crystal cell unit parameters for raw materials. In a, b, c and d cellulose unit-cell parameters. (e) interplanar spacing (d_{200}). (f) crystallites width (L_{200}).

The crystal cell-unit parameters a , b , c and γ (figure III-1a to 1d) are within the ranges reported in literature for cellulose I. For crystallite width (L_{200}), CTL has wider crystallites (55 Å) than the feedstocks richer in lignin and hemicelluloses (CCF, ESD, PSD, SIF, SCP and SCR), whose widths are in the range 23-29 Å. The wider crystals of CTL are a well-known fact and is thought to reflect cellulose co-crystallization enabled by the lack of hemicelluloses and lignin to keep cellulose crystals separated from one another (LEPPÄNEN et al., 2009). The interplanar spacing d_{200} have a variation between 3.91 to 4.00 Å, reflecting mainly a variation in the unit cell parameter a . The lowest d_{200} is found in CTL and is thought to reflect the larger crystal size L_{200} , which brings the spacing d_{200} closer to the $d_{200} = 3.86$ Å reported in the cellulose I β crystal structure (NISHIYAMA; LANGAN; CHANZY, 2002). The larger d_{200} comes from sugarcane biomass (SCP and SCR), which has been previously reported in literature (DRIEMEIER; SANTOS; BUCKERIDGE, 2012).

3.1.3 – Nanoscale porosity

The pore size distributions for the raw materials are presented in figure III-1. The errors bars are the standard deviation of duplicates. Pore size distribution for raw materials showed natural variability across the feedstocks of various biological origins.

Figure III - 2: Cumulative pore size distribution of raw lignocellulosic materials

The pore size distribution profiles show a division in two porosity groups, one with lower porosity and narrow differentiation between samples, SCR, PDS and ESD. The second one with more porous material and large spread in porosity between samples (SIF, SCP, CTL and CCF). This division can be explained by sample functionality in the samples. For the lower porosity they are materials that work as supporting element of the plant, they are more compact to provide the mechanical resistance necessary. The group of lower On the other hand, there are the high porosity materials, they work as storage, transport agent in the plant, this make these materials more malleable to attend the plant functionality. The figure III-3 present the relation of freezing bound water (FBW) versus lignin content in raw materials.

Figure III - 3: Cumulative freezing bound water (FBW) versus lignin content in raw materials and the linear fit between two parameters.

One interesting observation is the correlation between FBW (cumulative up to 200 nm) and lignin content shown in Figure III-3. There is a strong negative correlation ($r = -0.98$) between lignin content and FBW. This result agrees with the idea that lignin is a filler of the nanoscale pores of lignocellulosic biomass.

As reference materials for cellulose properties comparison the commercial celluloses (MCC, EKP and DIS), are standardized for use in the industry and can be obtained commercially in higher amounts. The pore size distributions of these materials are shown in figure III-4. Pore size distribution for commercial celluloses show that these materials are more porous than the raw lignocellulosic materials, with FBW cumulative up to pore diameter of

200 nm reaching more than 0.16 (g/g), compared to less than 0.16 (g/g) in the raw lignocellulosic materials (Figure III-2).

Figure III - 4: Pore size distribution of commercial cellulose.

3.2 – Variability induced by chemical processing

3.2.1 – Chemical composition of isolated celluloses

Chemical processes can change the compositional and structural variability beyond the natural variability of the feedstocks. Four distinct chemical treatments (LHW; ALK; LHW+ALK and ACT) were used to achieve this additional variability. These treatments (individually or combined) remove more than one lignocellulose component.

The removal of hemicelluloses and lignin in LHW treatment is consequence of hydrolysis reactions. The higher solubility of hemicelluloses in water leads to a more extensive solubilization of this polysaccharide and/or its oligomers. The solubilization of lignin sometimes is not possible and can allow the aggregation of the lignin fragments in nanometric globules (LANGAN et al., 2014) that can growth in large fragments. The aggregation of crystals by dehydration is another phenomenon as consequence of higher temperature (in our case in the liquid hot water treatment).

The ALK treatment is effective for both hemicelluloses and lignin removal. The alkaline medium and the action of hydroxide ions produce a more effective delignification when

compared to LHW treatment. The aqueous medium in ALK promotes both hydrolysis and dissolution of hemicelluloses.

The sequential application of LHW and ALK treatments is option to obtain enriched liquors in hemicelluloses and lignin, respectively after LHW and ALK steps. Considering the global yields, the sequential treatment produces dissolution of 50% – 60% of the original substrate masses.

The acetosolv process combines the acid hydrolysis of hemicelluloses and lignin with the capability of acetic acid to dissolve lignins. In general, the yields obtained in the acetosolv process are like that one is observed for ALK treatment.

The high temperatures ($>110^{\circ}\text{C}$) employed in these treatments allow the thermodynamic and kinetics barriers to be overcome to render the lignin and hemicelluloses removal from cell wall. The treated biomasses were bleached to remove residual lignin and produce a solid with higher cellulose content. The resulting bleached treated samples for here and further are chosen isolated celluloses. Figure III-5 shows the chemical composition of isolated celluloses from CCF, ESD, PSD, SCP, SCR and SIF, error bars are the standard deviation of duplicates.

Reported components in figure III-5 are cellulose, hemicelluloses and lignin. The lignin contents for cellulose pulps are the kappa number converted to percentage of lignin content. This conversion is necessary because the Klason method has limitation in the quantification of low-lignin contents in bleached materials. The kappa number is the method most used in paper and pulp industry to determine the degree of delignification in cellulose pulps. As the method reports the volume of potassium permanganate consumed in the determinations, the obtained values must be converted to grams of lignin / grams of bleached.

The effect of the bleaching in removal of lignin from non-treated raw materials can be seen in figure III-5a and 5b, which shows the decrease in lignin content.

Figure III-5c, 5d and 5f indicates the efficiency of hemicelluloses removal from hydrothermal treatments, either exclusive or sequentially combined with ALK, when compared with only ALK treatment (figure III-5d). The acetosolv treatment (figure III-5e) was also effective in removal of hemicelluloses.

Figure III - 5: Chemical composition of raw materials and raw bleached and the isolated celluloses. In the legend, treatments: LHW= liquid hot water, ALK = alkaline, ACT = acetosolv and LHW+AKL = liquid hot water + alkaline. The dot line shows 100%

The figure III-6 show the Kappa number for the cellulose pulps, the kappa number is related to Klason lignin, where Kappa number=10 correspond to 1,3% in lignin (TAPPI, 1999). The figure III-6 the Kappa number for all samples is below 10, this means for all cellulose pulps we have less than 1% in lignin. The low lignin content is important to prove that all properties measures in the celluloses pulps is a consequence of cellulose properties.

Figure III - 6: Kappa as function of cellulose content in isolated celluloses.

The cellulose concentrations in bleached samples are higher than in raw feedstocks because all compositions are reported as percentage of solid content. Since lignin and hemicelluloses are preferentially solubilized by the treatments, cellulose makes larger share of the remaining solid components.

3.2.3 – Chemical functionality by infrared spectroscopy

FTIR spectra of SCR materials are presented in figure III-7, as example of spectra to the all isolated celluloses. The spectra were subtracted from background (mean absorbance intensity at $3800\text{--}4000\text{ cm}^{-1}$) and then normalized by absorbance intensity at peak 1035 cm^{-1} .

FTIR spectra profiles for samples from different treatments exhibited overlapping bands mainly for the higher intensity bands from C-OH linkages at 1035 cm^{-1} and hydroxyl groups (OH) at $\sim 3500\text{ cm}^{-1}$.

The FTIR results are following the earlier discussion concerning lignin content in the samples, indicated by the reduction of the aromaticity band at 1515 cm^{-1} (figure III-7b). This is a second evidence (alongside the Kappa number results) that the cellulose pulps are free of lignin. FTIR spectra also showed another difference in the carbonyl region (figure III-7c). The raw samples showed absorption due to acetyl groups present in hemicelluloses. The presence of acetyl groups is identified by characteristics bands at $1740\text{--}1750\text{ cm}^{-1}$ (C=O stretching), 1368 cm^{-1} (C-H bending of acetyl group) and $1230\text{--}1240\text{ cm}^{-1}$ (acetyl group stretching) (LARKIN,

2011). This absorption is absent in the spectra of samples from LHW and ALK treatments. On the other hand, the acetosolv treatment results in partial acetylation of cellulose, as can be seen in figure III-7c.

Figure III - 7: (a) FTIR of isolated celluloses from sugarcane rind (SCR). (b) Region associated to lignin at 1515 cm^{-1} (aromatic region) (c). Region associated to acetyl group $1700\text{--}1750\text{ cm}^{-1}$.

An index of acetylation versus cellulose content is presented in Figure III-8. The index of acetylation is determined by the ratio of intensities at 1750 cm^{-1} ($\text{C}=\text{O}$ stretching) and 3400 cm^{-1} (O-H group) (HURTUBISE, 1962; CHANZY, 1984). This index of acetylation measured by FTIR is not a quantitative measurement but serves as a qualitative indication of the acetylation.

Figure III - 8: Index of acetylation versus cellulose content for all samples.

3.2.4 – Cellulose crystal parameters

X-ray diffraction patterns were analyzed by the Rietveld method using the CRAFS model (OLIVEIRA; DRIEMEIER, 2013; DRIEMEIER, 2014). Figure III-9 presents the diffractogram of cellulose pulps compared with the diffractogram of the raw materials.

Figure III - 9: X-ray diffraction patterns of various feedstocks and treatment conditions.

The first result from the diffraction patterns of Figure III-9 is that all cellulose pulps have the cellulose I polymorph, which is the same polymorph of the raw feedstocks. This allows

the comparison to be made quantitatively, based on the cellulose I crystal parameters extracted using CRAFS analysis.

Figure III-10 presents the crystal unit cell parameters (a , b , c and γ) for the isolated celluloses and the raw materials. The error bars are the standard deviation of duplicates. The crystal unit cell parameters show some. In this way, it is possible to correlate the variability in cellulose crystal parameters with the chemical purity of the isolated celluloses.

Figure III - 10: Unit cell parameters in the raw feedstocks and isolated celluloses. a) the a -axis length, b) b -axis length, c) the c -axis length and d) γ angle between a and b axis.

The first aspect to be noticed in Figure III-11 the trends between structure (crystal parameters) and composition (cellulose content). Noteworthy, there are variations of crystal width L_{200} and interplanar spacing d_{200} with cellulose content. We can see a clear trend of increasing L_{200} and decreasing d_{200} with increasing cellulose content. This effect was already reported for others samples (DRIEMEIER et al., 2011). The increasing of crystal width is the directly related to the effect of co-crystallization because the removal of hemicelluloses that surround cellulose crystals allowing that the interaction of two adjacent crystals become stronger than hemicellulose presence. The interplanar spacing (d_{200}) decrease is the directly consequence of the co-crystallization, because the close proximity of the crystals the interplane interaction resulting in approximation of cellulose chains.

Figure III-11: Cellulose crystal parameters, interplanar spacing (d_{200}) and crystal width (L_{200}) for the raw and isolated celluloses as a function of cellulose content. a) the interplanar spacing, b) crystal width.

3.2.5 – Nanometric porosity

The pore size distribution for isolated celluloses obtained by different treatments are present in figure III-12. The error bars are the standard deviation of duplicates. The pore size distribution is showed as cumulative freezing bound water for the samples.

Figure III - 12: Pores size distribution for isolated celluloses. Comparison with the raw and raw bleached. SCP = sugarcane pith, SCR = sugarcane rind, ESD = eucalyptus sawdust, PSD = pine sawdust, SIF = sisal fiber and CCF = coconut fiber.

The first point in figure III-12, is a clear increase in nanometric porosity for all isolated celluloses in relation to the raw lignocellulosic materials. This increase of nanometric porosity is consequence of two mechanisms. The first mechanism is the lignin removal from cell wall, reflecting the fact that the lignin present in the cell wall (secondary layer) is a filler of wall nanometric pores (voids). The removal of lignin makes pores accessible and so increase the nanometric porosity. The second mechanism is a consequence of increase the fragility of cell wall because treatments solubilize hemicelluloses and other structural molecules that help

keeping the wall structural integrity. Alongside this mechanisms there is delamination effect (separation of wall lamella) which is eased by the aforementioned delamination (MALONEY; PAULAPURO, 1999; FAHLÉN; SALMÉN, 2005; WANG et al., 2014; ZHAO; QI; LIU, 2017).

The second point is the effect of LHW treatment in the nanometric porosity. The isolated celluloses from LHW have less porosity than isolated celluloses from other treatments. This effect may be because of the cohesive action as result the co-crystallization of cellulose crystals undergoing during high temperature LHW conditions. The co-crystallization happens because the higher temperature promotes the dehydration of the cellulose crystals-crystals interfaces. Cellulose in wet state have the crystals surrounded by water, which keep adjacent crystals separated from one another. Rising the temperature, water leaves the crystal-crystal interface and allows the co-crystallization phenomena to happen (LEPPÄNEN et al., 2009; DRIEMEIER et al., 2011; NISHIYAMA et al., 2014). The XRD results of isolated celluloses evidence the effect of co-crystallization (increasing crystal width L_{200}) after LHW treatment. This cohesive action during LHW treatment is a disadvantage in biorefinery applications because the aggregation reduces cellulose accessibility.

The third interesting result is the porosity of cellulose pulps for the same chemical treatment. There is no clear trend between source of cellulose and porosity.

The pore area of cellulose pulps is presented in figure III-13, the error bar is the standard deviation of duplicates. The pore area showed is the total sum of pore area for pores bigger than 10 nm. This pore area result shows an increase of the pore area as function of chemical treatment, with the alkaline (ALK) treatment promoting the biggest increase of pore area, followed by acetosolv (ACT) treatment. Liquid hot water (LHW) and liquid hot water + alkaline (LHW+AKL) have the smallest pore area.

Figure III - 13: Pore area > 10 nm versus cellulose content in the commercial isolated celluloses.

Figure III-14 shows the value of the non-freezing bound water (NFBW) for isolated celluloses in comparison to the raw materials. The error bars are standard deviation of duplicates. The NFBW do not show any trend as function of cellulose source or treatment, which can be attributed to the high uncertainty of this determination.

Figure III - 14: NFBW versus cellulose content in the isolated celluloses.

3.2.6 – Moisture sorption

Figure III-15 shows the moisture desorption and sorption isotherms for cellulose isolated from sugarcane rind. The sorption and desorption isotherms are fitted by Hailwood-Horrobin sorption model (HH-model).

Figure III - 15: Moisture desorption and sorption isotherms for SCR pulps. The empty square stands for the desorption branch and the filled squares the sorption. The lines are the fitted curves obtained from HH-model.

The values showed in figure III-14 are the experimental data (dots) and the fit (line) with the HH model. The HH model fit shows good agreement with the experimental data, with R-square ~ 0.999 for most the samples analyzed up to RH < 0.8 . For RH > 0.8 the fits deviate from the experimental data.

Measured and fitted isotherms from celluloses isolated from sugarcane rind in figure III-14 exemplify the isotherms in all the samples. The first result is that all isotherms are from type II (LOWELL et al., 2012), with the desorption isotherm always over the sorption isotherms. This effect is known as hysteresis. Hysteresis can be bigger or smaller depending on

the samples. The hysteresis is a result of the two mechanisms: capillary condensation and/or structure conformation.

The HH-model describes the isotherms with three parameters: monolayer water (m_0), free energy of monolayer hydration (ΔG_h) and free energy of dissolved water (ΔG_d). These parameters obtained from the sorption and desorption data, are presented in figure III-16. The Hailwood-Horrobin model

Figure III - 16: Parameters obtained from the Hailwood-Horrobin model (HH-model) applied to moisture desorption and sorption isotherms. In a monolayer water m_0 . In b d free energy of dissolving water ΔG_d . In c free energy of monolayer hydration ΔG_h .

In figure III-16 the first consideration of m_0 is that for all the isolated celluloses submitted to LHW and LHW+ALK treatments have relatively lower values. This seems to be correlated to the lower porosity of these isolated celluloses (see figure III-14). This relation may be understood as an overall tendency for lower hydration spanning across length scales, from monolayer water to 200 nm pores.

3.2.7 – Multivariate insights from isolate celluloses

Figure III-17 shows the water monolayer (m_0) from sorption isotherms of HH-model (figure III-15) versus the reciprocal of crystal width ($1/L_{200}$) obtained from XRD.

Figure III - 17: Monolayer water m_0 versus reciprocal of crystal width ($1/L_{200}$).

The correlation m_0 vs $1/L_{200}$ was made for sorption m_0 data only, because desorption m_0 is highly inflected by water in pores. The trend in m_0 vs $1/L_{200}$ is a good and positive correlation ($r=0.91$) and fit with $R^2=0.83$. This trend was observed in a previous work (DRIEMEIER; BRAGATTO, 2013) and served to demonstrate that crystal width has a dominant role on the hydration of celluloses isolated from plants.

Another correlation worth evaluating is m_0 and hemicelluloses content, presented in figure III-18. hemicelluloses are usually assumed to have a key role on hydration,

The third major correlation is the freezing bound water (FBW) and the crystal width (L_{200}), present in figure III-17. Noteworthy, FBW is measured as function of pore diameter, so the correlations can be calculated for each pore diameter.

The correlation of L_{200} and FBW shows an interesting and unexpected behavior as function of pore diameter. The correlation is positive for pores $< 4\text{nm}$ and then becomes negative for larger pores. Figure III-18 shows the relation of FBW versus crystals width for selected ranges of pore diameters, exemplifying the trends with opposing signs in correlations.

Figure III - 18: Correlations factors between freezing bound water (FBW) and crystal width (L_{200}) versus the pore diameter.

Figure III - 19: Relation of FBW versus crystals width L_{200} in distinct ranges of pore diameter.

3.2.7.1 – Principal components analysis (PCA)

PCA promotes a dimension reduction of the data set, providing a simple way to interpret multivariate data sets with highly correlated variables. We analyzed a data set made of isolated celluloses described by four variables: m_0 , $1/L_{200}$, cellulose and hemicelluloses contents and each line are different sample. Table III-3 presents the PCA variances of the data set, showing that 88% of the variance is contained in the first two PCs.

Table III - 2: Percentage of total variance in principal component analysis.

PCA	Variance %	Cumulative
PC1	70.94	70.94
PC2	17.14	88.08
PC3	9.93	98.01
PC4	1.99	100.00

Figure III-20 presents the loadings of the variables in the PC1xPC2 space. The length of each arrow stands for the fraction of feature variance represented by the reduced dimensionality. The circle of unitary radius serves as reference. The cosine of the angle between two arrows estimates the correlation between the variables, with parallel arrows having correlation 1, anti-parallel for correlation -1, and perpendicular for correlation 0. In figure III-19, the arrows corresponding to hemicelluloses and cellulose are anti-parallel, which means they have -1 correlation. This is expected because they are complementary components in isolated celluloses: the increase of one is the decreasing the other.

Hydration monolayer (m_0) arrow is between of $1/L_{200}$ and hemicellulose content, which shows that the monolayer water is influenced by these two parameters, from structure and composition.

Figure III - 20: Factor loadings of cellulose content, hemicelluloses content, monolayer hydration m_0 , and reciprocal crystal width $1/L_{200}$ for the set of celluloses isolated from plants.

Figure III-21 presents the scores plot in the PC1×PC2 plane for all samples. This plot presents the samples distribution variance in relation to the PC1 and PC2.

Figure III - 21: Relation between the scores and PC1 and PC2

The results displayed in figure III-21 show that the samples spread for all the variance range. This is positive, because it proves we achieved our goal of generating a highly variable set of celluloses isolated from plants. The fact that the samples are spread along the PC1 axis more than the PC2 axis is a consequence of most of the variance belonging to PC1.

3.3 – Production of cellulose nanocrystals

3.3.1 - Qualitative observations in CNC production

The initial efforts for production of CNCs have shown some drawbacks, as we were unable to produce CNCs from some of the isolated celluloses. This was particularly the case of celluloses resulting from the acetosolv treatment. During the treatment with concentrated sulfuric acid to prepare CNC, all the acetosolv samples were strongly oxidized, producing black suspensions. This shows that the condition employed for these samples were too drastic. The reason is still unclear, but it seems plausible that the isolated celluloses resulting from acetosolv treatment were more susceptible to the acid attack, perhaps because of lower level of cellulose co-crystallization due to the higher level of acetylation. Fortunately, we could successfully produce CNCs from all other isolated celluloses, which allowed quantitative characterizations.

3.3.2 – Mass balance in cellulose nanocrystals production

CNCs were successfully obtained from commercial celluloses (DIS, EKP and MCC) and from the celluloses isolated from ESD, SCR and SCP using three different chemical treatments (LHW, ALK and LHW+ALK). Figure III-22 shows the CNC yields, monosaccharide production, and residual mass of unhydrolyzed solids quantified in CNC production. The error bars are standard deviations from duplicates. In Figure III-22, the first observation is the amount of non-quantified products (see the distance from 100% mass balance), which results from the experimental difficulties in the production of CNC. It is worth recalling that CNC production requires a hydrolysis step, when the mixture between the acid and the cellulose substrate must be made carefully to avoid excessive cellulose degradation or low hydrolysis degree that does not lead to CNC. Another big challenge is to separate the reaction products (CNC, monosaccharides, and non-hydrolysate solid) because mixtures of these components always exist during the whole process. The sugar content is significantly reduced by dialysis performed to remove the excess of sulfuric acid. Another source of difficulty is the separation of CNC from non-hydrolysate solids. The filtration process is normally slow and can leave to some aggregation of the CNC's and the non-hydrolysate solids. The optimizing of the experimental procedures should reduce the uncertainties and improve the yields in the CNC production. This set of challenges cause high uncertainty and experimental

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limitations in yield determination, which helps explaining the scarcity of yield results in the literature.

The second result we can learn from figure III-22 is that the CNC yield is in a narrow range for the celluloses isolated in this work (13-19%) as well as for the commercial celluloses (20-27%). No clear trend comparing the CNC yields of the different substrates, which may result from high uncertainties in yield measurements. The third result is the higher monosaccharides production for cellulose pulps from LHW+ALK chemical treatment when compared to others chemical treatments. At this moment we do not have an explanation for this result.

Figure III - 22: Mass balance of CNC production by acid hydrolysis with sulfuric acid. The dashed line shows the reference of 100% mass closure.

3.3.3– Cellulose nanocrystals morphology

The cellulose nanocrystals morphology was determined by imaging with AFM followed and image analysis to determine length (L), width (D) and aspect-ratio (L/D). Figure III-23 presents selected AFM images of CNC obtained from commercial pulps (DIS, EKP and MCC).

Figure III - 23: AFM images of commercial cellulose pulps. In a-DIS, b-EKP and c-MCC.

From figure III-23 it is possible to see the well-known CNC morphology of elongated particles looking like needles. The first observation is that some agglomeration of CNC is observed in figure III-23 (c). This agglomeration is a common issue in imaging CNC. Nevertheless, in the same image one can also see individual particles. Figure III-24 shows the histograms of length and width of CNC for dissolving pulp (in a and b), Eucalyptus Kraft Pulp (in c and d) and microcrystalline cellulose (in e and f).

Figure III - 24: Histogram of CNC dimensions measures form AFM. L= length and D= width. For commercial celluloses. In a and b from DIS, in c and d from EKP and in e and f from MCC.

Figure III - 25: Histogram of CNC dimensions measures form AFM. L= length and D= width. For the celluloses isolated from hydrothermal process (LHW)In a and b from ESD, in c and d from SCR and in e and f from SCP.

Figure III - 26: Histogram of CNC dimensions measures form AFM. L= length and D= width. For the celluloses isolated from alkaline process (ALK). In a and b from ESD, in c and d from SCR and in e and f from SCP

Figure III - 27: Histogram of CNC dimensions measures form AFM. L= length and D= width. For the celluloses isolated from liquid hot water followed alkaline process (LHW+ALK). In a and b from ESD, in c and d from SCR and in e and f from SCP.

Table III-3 shows the values L, D and L/D for all CNC produced. The deviations were calculated from the at least 20 particles. Some effect of aggregation of the CNC particles generate large dispersions in the measurements, as shown in table III- 3.

Table III - 3: Morphology dimensions of CNC from difference sources of cellulose pulp and chemical treatments.

Treatment	Cellulose pulp	L (nm)	D (nm)	L/D
Commercial pulps	DIS	150 ± 39	5 ± 3	29
	EKP	171 ± 53	4 ± 2	43
	MCC	179 ± 35	7 ± 2	26
LHW	ESD	280 ± 74	5 ± 2	56
	SCR	210 ± 45	6 ± 2	35
	SCP	319 ± 71	8 ± 3	40
ALK	ESD	282 ± 65	5 ± 2	59
	SCR	214 ± 66	6 ± 2	36
	SCP	241 ± 88	6 ± 2	44
LHW+ALK	ESD	251 ± 83	7 ± 2	36
	SCR	223 ± 62	7 ± 2	34
	SCP	287 ± 57	8 ± 5	36

L = length, D = width, L/D=aspect ratio. LHW = liquid hot water, ALK =alkaline and LHW+ALK = liquid hot water+ alkaline. DIS = Dissolving pulp, EKP = Eucalyptus kraft pulp, MCC = Microcrystalline cellulose (Avicel), ESD = Eucalyptus sawdust, SCR =Sugarcane rind, SCP = sugarcane pith.

In table III-3, the commercial pulps resulted in the shorter CNCs, with lengths between 150 and 179 nm. On the other hand, LHW samples produce the longest CNCs, between 210 and 319 nm. The ALK celluloses lead to CNCs with 214 to 282 nm long, with similar ranges observed for CNCs from LHW+ALK substrates.

The CNC samples produced from sugarcane fractions (SCR and SCP) present values in agreement with literature, Teixeira *et al* and Kumar *et al* reported values of length (L) from 250 to 480 nm and width (D) from 4 to 60 nm (TEIXEIRA et al., 2011; KUMAR et al., 2014). From our results, we can say there is no significant difference between SCR and SCP CNCs dimensions.

The figure III-23, is possible to see well-known CNC structure, allonged particles looking like needles. The first observation is that are some agglomeration of CNC in c, corresponding to MCC, this agglomeration common issue in making images from CNC, but is also possible to see individual particles. The figure III-24 show the histograms of length and

width of CNC of dissolving pulp (in a and b), Eucalyptus Kraft Pulp (in c and d) and microcrystalline cellulose (in e and f).

3.3.4 – Cellulose nanocrystals crystals parameters

The obtained CNCs were also analyzed by XRD. However, before evaluating cellulose I crystal parameters, we must note that the diffraction patterns presented several features that deviate from the characteristic cellulose I diffraction signal. In some XRD patterns show characteristic signals of the cellulose II polymorph, in some cases mixed with cellulose I. The formation of cellulose II is a consequence of dissolution of some fractions of cellulose during acid hydrolysis followed by precipitation as cellulose II during the dilution step of CNC production. Figure III-28 shows diffractograms of the CNC samples that present possible mixtures of cellulose I with cellulose II. The diffractograms show the problems we had during CNC production. For such diffractograms the model fits are poor because CRAFS is designed to fit data of cellulose I only. This makes the values of crystals parameters for these materials inaccurate in some extension. This impedes the comparison between crystal parameters of the CNC samples.

Figure III - 28: X-ray diffractograms of CNC's from: (a) MCC, (b) ESD alkaline pulp, (c) SCR liquid hot water pulp and (d) SCR alkaline pulp.

3.3.5 – Role of feedstock variability in CNC production

We observed a significant correlation between the CNCs aspect-ratio (L/D) and the pore area of the cellulose substrates obtained in different treatments, as shown in figure III-29.

Figure III - 29: Cellulose nanocrystal aspect-ratio (L/D) vs. pore area for isolated cellulose from different sources and treatments.

In figure III-29, the aspect-ratio (L/D) have positive correlation to pore area ($r = 0.70$). The aspect-ratio is a more interesting parameter than length and diameter, because is the principal factor that lead the application of CNC in composites. Moreover, figure III-30 shows a correlation between L_{200} from CNC and its correspondent isolated cellulose sources.

Figure III - 30: Correlation between crystal width from CNC versus crystal width from isolated celluloses.

There is a good correlation between the two parameters, especially if we discard the upper left 4 data points that seem to be dislocated. These outlying points can be traced to the diffraction patterns with interferences of cellulose II, where CRAFS parameters are not meaningful, as discussed earlier (figure III-28).

Although all the difficulties found in producing CNC, correlations between CNC properties and isolated celluloses was possible. The correlations show some trends in the between the parameters, showing the properties of the source materials continue in the CNCs. The mass balance of CNC production provide a good indication the possible products during the CNC production.

Chapter IV - Conclusions

The general objective of this Thesis was the investigation of the variability in plant cellulose, with emphasis on nanostructure parameters, and then additional investigation on how this variability affects the production of cellulose nanocrystals.

This objective was achieved, with the remark that, in the pursuit of our first specific objective, we obtained a wide set of celluloses isolated from plants, with variability originating from diverse biological sources (cotton linter, coconut fiber, sisal fiber, eucalyptus sawdust, pine sawdust, sugarcane rind and sugarcane pith) combined with variable chemical processing conditions (acetosolv, liquid hot water, alkaline, and liquid hot water + alkaline). Commercial celluloses were also studied to further expand the sample set. Variability was observed in all the measured features of the isolated celluloses: contents of macromolecular components (cellulose, hemicelluloses and lignin), chemical functionality, cellulose crystal parameters, nanoscale porosity, and moisture sorption behavior.

Across the broad set of celluloses isolated from plants, we observed several important correlations, which suggest underlying mechanisms linking the correlated variables. Selected correlations are worth highlighting. Cellulose crystal width (L_{200} , determined by X-ray diffraction) increases as lignin and hemicelluloses are removed, reflecting a phenomenon known as cellulose co-crystallization.

Nanoscale porosity also increases after chemical treatment for all types of biomass, likely a combined result of removing lignin (a pore filler) and opening of walls by delamination enabled by fragilization due to component removal. The correlation between freezing bound water and crystal width showed an interesting swing, from positive to negative, as function of pore size in the 1-200 nm range. This result indicates crystal width affects cellulose hydration in distinct ways as one considers different length scales of the cellulose hierarchical structures. The correlations between crystal width, monolayer hydration (inferred from moisture sorption) and contents of cellulose and hemicelluloses are also revealing.

Principal component analysis shows both crystal width and hemicelluloses contents have comparable role on monolayer hydration, and this role on hydration is expected to impact acid hydrolysis in production of cellulose nanocrystals.

Our second specific objective, production of celluloses nanocrystals, was also achieved. The cellulose nanocrystals were characterized and results revealed nanocrystal variabilities generated under identical production conditions, which can be attributed to the variability of

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the cellulose substrates used in nanocrystal production. That brings us to the third specific objective, to relate nanocrystal properties with the properties of the cellulose substrates used to generate them. The nanocrystal yields (13-27%) obtained in this work follows typical literature data for unoptimized hydrolysis process. In addition to yields, we produced mass balances counting also the non-hydrolyzed solid and the sugars resulting from hydrolysis.

As for the morphological properties of cellulose nanocrystals, our results reproduced typical literature values, with length in the range of 100-400 nm and mean diameter

Resume this work was able to produce a group of data of nanostructural parameters from different celluloses. Correlations between the nanostructural parameters provide a new view of the nanostructure of celluloses and how this can affect the cellulose nanocrystal production. The data produce can help future researches as start point for optimization process or deeper investigations in some parameters.

References

- ABITBOL, T.; KLOSER, E.; GRAY, D. G. Estimation of the Surface Sulfur Content of Cellulose Nanocrystals Prepared by Sulfuric Acid Hydrolysis. **Cellulose**, v. 20, n. 2, p. 785–794, 2013.
- AGBOR, V. B.; CICEK, N.; SPARLING, R.; BERLIN, A.; LEVIN, D. B. Biomass Pretreatment: Fundamentals toward Application. **Biotechnology Advances**, v. 29, n. 6, p. 675–685, 2011.
- AKGUL, M.; KIRCI, H. An Environmentally Friendly Organosolv (Ethanol-Water) Pulping of Poplar Wood. **Journal of Environmental Biology**, v. 30, n. 5, p. 735–740, 2009.
- AKHBARI, M.; ZAHIRI, A.; BASSAM, S. J. E. Optimization of Parameters Influencing Mercerization Using the RSM Method in Order to Increase the Tensile Strength of Mercerized Yarn. **Fibres and Textiles in Eastern Europe**, v. 94, n. 5, p. 30–35, 2012.
- ALVIRA, P.; TOMÁS-PEJÓ, E.; BALLESTEROS, M.; NEGRO, M. J. Pretreatment Technologies for an Efficient Bioethanol Production Process Based on Enzymatic Hydrolysis: A Review. **Bioresource Technology**, v. 101, n. 13, p. 4851–4861, Jul. 2010.
- ATALLA, R. H.; VANDERHART, D. L. Native Cellulose: A Composite of Two Distinct Crystalline Forms. **Science (New York, N.Y.)**, v. 223, n. 4633, p. 283–285, 1984.
- BAKKER, H. **Sugar Cane Cultivation and Management**. United kingdom: Springer, 1926.
- BECK-CANDANEDO, S.; ROMAN, M.; GRAY, D. G. Effect of Reaction Conditions on the Properties and Behavior of Wood Cellulose Nanocrystal Suspensions. **Biomacromolecules**, v. 6, n. 2, p. 1048–1054, 2005a.
- BECK-CANDANEDO, S.; ROMAN, M.; GRAY, D. G. Effect of Reaction Conditions on the Properties and Behavior of Wood Cellulose Nanocrystal Suspensions. **Biomacromolecules**, v. 6, n. 2, p. 1048–1054, 2005b.
- BECK, S.; MÉTHOT, M.; BOUCHARD, J. General Procedure for Determining Cellulose Nanocrystal Sulfate Half-Ester Content by Conductometric Titration. **Cellulose**, v. 22, n. 1, p. 101–116, 2015.
- BELGACEM, M. N.; GANDINI, A. **Monomers , Polymers and Composites**. First ed. [s.l.] Elsevier, 2008.
- BERG, H. CELLULOSE. In: **Encyclopedia of Food Sciences and Nutrition**. Cambridge: Elsevier, 2003. p. 998–1007.
- BHIMTE, N. a; TAYADE, P. T. Evaluation of Microcrystalline Cellulose Prepared from Sisal Fibers as a Tablet Excipient: A Technical Note. **AAPS PharmSciTech**, v. 8, n. 1, p. 8, 2007.

- BOBLETER, O. Hydrothermal Degradation and Fractionation of Saccharides and Polysaccharides. In: DUMITRIU, S. (Ed.). **Polysaccharides: Structural Diversity and Functional Versatility**. 2. Ed. p. 1204.
- BONDESON, D.; MATHEW, A.; OKSMAN, K. Optimization of the Isolation of Nanocrystals from Microcrystalline Cellulose by Acid Hydrolysis. **Cellulose**, v. 13, n. 2, p. 171–180, 2006.
- BRITO, B. S. L.; PEREIRA, F. V.; PUTAUX, J. L.; JEAN, B. Preparation, Morphology and Structure of Cellulose Nanocrystals from Bamboo Fibers. **Cellulose**, v. 19, n. 5, p. 1527–1536, 2012.
- BRITO, J. O.; SILVA, F. G.; LEÃO, M. M.; ALMEIDA, G. Chemical Composition Changes in Eucalyptus and Pinus Woods Submitted to Heat Treatment. **Bioresource Technology**, v. 99, n. 18, p. 8545–8548, 2008.
- BU, L.; HIMMEL, M. E.; CROWLEY, M. F. The Molecular Origins of Twist in Cellulose I-Beta. **Carbohydrate Polymers**, v. 125, p. 146–152, Jul. 2015.
- CAMARERO ESPINOSA, S.; KUHN, T.; FOSTER, E. J.; WEDER, C. Isolation of Thermally Stable Cellulose Nanocrystals by Phosphoric Acid Hydrolysis. **Biomacromolecules**, v. 14, n. 4, p. 1223–1230, 2013.
- CANETTIERI, E. V.; ROCHA, G. J. de M.; CARVALHO, J. A. De; SILVA, J. B. de A. e. Optimization of Acid Hydrolysis from the Hemicellulosic Fraction of Eucalyptus Grandis Residue Using Response Surface Methodology. **Bioresource Technology**, v. 98, n. 2, p. 422–428, 2007.
- CAZACU, G.; POPA, V. I. Blends and Composites Based on Cellulose Materials. **Polysaccharides: Structural Diversity and Functional Versatility**, 2005.
- CHANZY, H. Ultrastructural Aspects of the Swelling of Cellulose. **Papier**, v. 38, p. 582, 1984.
- CHEN, L.; WANG, Q.; HIRTH, K.; BAEZ, C.; AGARWAL, U. P.; ZHU, J. Y. Tailoring the Yield and Characteristics of Wood Cellulose Nanocrystals (CNC) Using Concentrated Acid Hydrolysis. **Cellulose**, v. 22, n. 3, p. 1753–1762, Jun. 2015.
- CHIARAMONTI, D.; PRUSSI, M.; FERRERO, S.; ORIANI, L.; OTTONELLO, P.; TORRE, P.; CHERCHI, F. Review of Pretreatment Processes for Lignocellulosic Ethanol Production, and Development of an Innovative Method. **Biomass and Bioenergy**, v. 46, p. 25–35, 2012.
- CHUM, H. L.; JOHNSON, D. K.; BLACK, S.; BAKER, J.; GROHMANN, K.; SARKANEN, K. V. Organosolv Pretreatment for Enzymatic Hydrolysis of Poplars: I. Enzyme Hydrolysis of Cellulosic Residues. **Biotechnology and Bioengineering**, v. 31, p. 643–649, 1988.
- CONAB, C. N. de A. **Acompanhamento da safra brasileira de cana-de-açúcar-Safra 2016/17-Quarto levantamento**.
-

- CONLEY, K.; GODBOUT, L.; WHITEHEAD, M. A. (Tony); VAN DE VEN, T. G. M. Origin of the Twist of Cellulosic Materials. **Carbohydrate Polymers**, v. 135, p. 285–299, Jan. 2016.
- CORRÊA, A. C.; DE TEIXEIRA, E. M.; PESSAN, L. A.; MATTOSO, L. H. C. Cellulose Nanofibers from Curaua Fibers. **Cellulose**, v. 17, n. 6, p. 1183–1192, 2010.
- CRUZ, V. M. V.; DIERIG, D. A. **Industrial Crops-Breeding for BioEnergy and Bioproducts**. Springer, 1996. v. 5.
- DA CRUZ, S. H.; NICHOLS, N. N.; DIEN, B. S.; SAHA, B. C.; COTTA, M. A. Hydrothermal Pretreatment of Sugarcane Bagasse Using Response Surface Methodology Improves Digestibility and Ethanol Production by SSF. **Journal of Industrial Microbiology and Biotechnology**, v. 39, n. 3, p. 439–447, 2012.
- DAUD, J. B.; LEE, K.-Y. Surface Modification of Nanocellulose. In: **Handbook of Nanocellulose and Cellulose Nanocomposites**. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2017. p. 101–122.
- DONG, X. U. E. M. I. N.; REVOL, J.-F.; GRAY, D. G. Effect of Microcrystallite Preparation Conditions on the Formation of Colloid Crystals of Cellulose. **Cellulose**, v. 5, n. 1, p. 19–32, 1998.
- DRIEMEIER, C. Two-Dimensional Rietveld Analysis of Celluloses from Higher Plants. **Cellulose**, v. 21, n. 2, p. 1065–1073, 2014.
- DRIEMEIER, C.; BRAGATTO, J. Crystallite Width Determines Monolayer Hydration across a Wide Spectrum of Celluloses Isolated from Plants. **Journal of Physical Chemistry B**, v. 117, n. 1, p. 415–421, 2013.
- DRIEMEIER, C.; CALLIGARIS, G. A. Theoretical and Experimental Developments for Accurate Determination of Crystallinity of Cellulose i Materials. **Journal of Applied Crystallography**, v. 44, n. 1, p. 184–192, 2011.
- DRIEMEIER, C.; FRANCISCO, L. H. X-Ray Diffraction from Faulted Cellulose I Constructed with Mixed I α -I β Stacking. **Cellulose**, v. 21, n. 5, p. 3161–3169, 2014.
- DRIEMEIER, C.; MENDES, F. M.; OLIVEIRA, M. M. de. Dynamic Vapor Sorption and Thermoporometry to Probe Water in Celluloses. **Cellulose**, v. 19, n. 4, p. 1051–1063, 2012.
- DRIEMEIER, C.; PIMENTA, M. T. B.; ROCHA, G. J. M.; OLIVEIRA, M. M. de; MELLO, D. B.; MAZIERO, P.; GONÇALVES, A. R. Evolution of Cellulose Crystals during Prehydrolysis and Soda Delignification of Sugarcane Lignocellulose. **Cellulose**, v. 18, n. 6, p. 1509–1519, 2011.
- DRIEMEIER, C.; SANTOS, W. D.; BUCKERIDGE, M. S. Cellulose Crystals in Fibrovascular Bundles of Sugarcane Culms: Orientation, Size, Distortion, and Variability. **Cellulose**, v. 19, n. 5, p. 1507–1515, 2012.
-

- DUAN, C.; LONG, Y.; LI, J.; MA, X.; NI, Y. Changes of Cellulose Accessibility to Cellulase Due to Fiber Hornification and Its Impact on Enzymatic Viscosity Control of Dissolving Pulp. **Cellulose**, v. 22, n. 4, p. 2729–2736, 2015.
- DUCHEMIN, B. J. C. Mercerisation of Cellulose in Aqueous NaOH at Low Concentrations. **Green Chem.**, v. 17, n. 7, p. 3941–3947, 2015.
- DUFRESNE, A. **Nanocellulose: From Nature to High Performance Tailored Materials**. First ed. De Gruyter, 2012.
- DUFRESNE, A.; BELGACEM, M. N. Cellulose-Reinforced Composites: From Micro-to Nanoscale. **Polímeros Ciência e Tecnologia**, v. 20, n. 1, p. 1–10, 2010.
- EBRINGEROVÁ, A.; HROMÁDKOVÁ, Z.; HEINZE, T. Hemicellulose. In: **Polysaccharides I**. Berlin/Heidelberg: Springer-Verlag, 2005. p. 1–67.
- EICHHORN, S. J. Cellulose Nanowhiskers: Promising Materials for Advanced Applications. **Soft Matter**, v. 7, n. 2, p. 303, 2011.
- EK, M.; GELLERSTEDT, G.; HENRIKSSON, G. **Wood Chemistry and Wood Biotechnology**.
- ELSAKHAWY, M.; HASSAN, M. L.; EL-SAKHAWY, M.; HASSAN, M. L.; ELSAKHAWY, M.; HASSAN, M. L.; EL-SAKHAWY, M.; HASSAN, M. L. Physical and Mechanical Properties of Microcrystalline Cellulose Prepared from Agricultural Residues. **Carbohydrate Polymers**, v. 67, n. 1, p. 1–10, 2007.
- FAHLÉN, J.; SALMÉN, L. Pore and Matrix Distribution in the Fiber Wall Revealed by Atomic Force Microscopy and Image Analysis. **Biomacromolecules**, v. 6, n. 1, p. 433–438, 2005.
- FAN, J.; LI, Y. Maximizing the Yield of Nanocrystalline Cellulose from Cotton Pulp Fiber. **Carbohydrate Polymers**, v. 88, n. 4, p. 1184–1188, 2012.
- FAO. **FAOSTAT-Sisal**. www.fao.org/faostat/en/#search/sisal. Acesso em: 26 feb. 2018a.
- FAO. **FAOSTAT-Coconut**. www.fao.org/faostat/en/#search/Coconuts. Acesso em: 27 feb. 2018b.
- FAO. **FAOSTAT-Cotton linter**. [www.fao.org/faostat/fr/#search/cotton linter](http://www.fao.org/faostat/fr/#search/cotton%20linter). Acesso em: 28 feb. 2018c.
- FAO. **FAOSTAT-Wood products**. www.fao.org/faostat/en/#data/FO. Acesso em: 6 mar. 2018d.
- FAO. **Chemical pulp**. [www.fao.org/faostat/en/#search/kraft pulp](http://www.fao.org/faostat/en/#search/kraft%20pulp). Acesso em: 21 mar. 2018e.
- FAVIER, V.; CANOVA, G. R.; CAVAILLÉ, J. Y.; CHANZY, H.; DUFRESNE, A.; GAUTHIER, C. Nanocomposite Materials Form Latex and Cellulose Whiskers. **Polymers for Advanced Technologies**, v. 6, n. 5, p. 351–355, 1995.
-

- FAVIER, V.; CAVAILLE, J. Y.; CANOVA, G. R.; SHRIVASTAVA, S. C. Mechanical Percolation in Cellulose Whisker Nanocomposites. **Polymer Engineering Science**, v. 37, n. 10, p. 1732–1739, 1997.
- FENGEL, D.; WEGENER, G. **Wood: Chemistry, Ultrastructure, Reactions**. Walter De Gruyter Incorporated, 1989.
- FERNANDES DINIZ, J. M. B.; GIL, M. H.; CASTRO, J. A. A. M. Hornification - Its Origin and Interpretation in Wood Pulps. **Wood Science and Technology**, v. 37, n. 6, p. 489–494, 2004.
- FRENCH, A. D. Glucose, Not Cellobiose, Is the Repeating Unit of Cellulose and Why That Is Important. **Cellulose**, v. 24, n. 11, p. 4605–4609, 2017.
- FU, F.; YANG, Q.; ZHOU, J.; HU, H.; JIA, B.; ZHANG, L. Structure and Properties of Regenerated Cellulose Filaments Prepared from Cellulose Carbamate – NaOH/ZnO Aqueous Solution. **ACS Sustainable Chemistry & Engineering**, 2014.
- GARCIA-GARCIA, D.; LOPEZ-MARTINEZ, J.; BALART, R.; STRÖMBERG, E. Reinforcing Capability of Cellulose Nanocrystals Obtained from Pine Cones in a Biodegradable Poly (3-Hydroxybutyrate)/ Poly (ϵ -Caprolactone) (PHB / PCL) Thermoplastic Blend. **European Polymer Journal**, v. 104, n. March, p. 10–18, 2018.
- GARROTE, G.; DOMÍNGUEZ, H.; PARAJÓ, J. C. Hydrothermal Processing of Lignocellulosic Materials. **Holz als Roh- und Werkstoff**, v. 57, n. 3, p. 191–202, 1999.
- GERALD, K. **Raw Material for Pulp** *Handbook of Pulp*: Wiley Online Books., 30 Jan. 2008.
- GHATAK, H. R. Biorefineries from the Perspective of Sustainability: Feedstocks, Products, and Processes. **Renewable and Sustainable Energy Reviews**, v. 15, n. 8, p. 4042–4052, 2011.
- GOLDEMBERG, J. The Brazilian Biofuels Industry. **Biotechnology for biofuels**, v. 1, n. 1, p. 6, 2008.
- GURGEL, L. V. A. **Hidrólise Ácida de Bagaço de Cana-de-Açúcar: Estudo Cinético de Sacarificação de Celulose para Produção de Etanol**. 2010. Universidade de São Paulo, 2010.
- HABIBI, Y. Key Advances in the Chemical Modification of Nanocelluloses. **Chem. Soc. Rev.** **Chem. Soc. Rev.**, v. 43, n. 43, p. 1519–1542, 2014.
- HAILWOOD, A. J.; HORROBIN, S. Absorption of Water by Polymers: Analysis in Terms of a Simple Model. **Transactions of the Faraday Society**, v. 42, n. 0, p. B084–B092, 1946.
- HALONEN, H.; LARSSON, P. T.; IVERSEN, T. Mercerized Cellulose Biocomposites: A Study of Influence of Mercerization on Cellulose Supramolecular Structure, Water Retention Value and Tensile Properties. **Cellulose**, v. 20, n. 1, p. 57–65, 2013.
- HAYASHI, T.; OGAWA, K.; MITSUISHI, Y. Characterization of the Adsorption of Xyloglucan to Cellulose. **Plant and Cell Physiology**, v. 35, n. 8, p. 1199–1205, 1994.
-

- HAYASHI, T.; TAKEDA, T.; OGAWA, K.; MITSUISHI, Y. Effects of the Degree of Polymerization on the Binding of Xyloglucans to Cellulose. **Plant & cell physiology**, v. 35, n. 6, p. 893–9, 1994.
- HIMMEL, M. E.; DING, S.-Y.; JOHNSON, D. K.; ADNEY, W. S.; NIMLOS, M. R.; BRADY, J. W.; FOUST, T. D. Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production. **Science (New York, N.Y.)**, v. 315, n. 5813, p. 804–807, 2007.
- HOI, L. W. S.; MARTINCIGH, B. S. Sugar Cane Plant Fibres: Separation and Characterisation. **Industrial Crops and Products**, v. 47, p. 1–12, 2013.
- HU, F.; RAGAUSKAS, A. Pretreatment and Lignocellulosic Chemistry. **Bioenergy Research**, v. 5, n. 4, p. 1043–1066, 2012.
- HURTUBISE, F. G. The Analytical and Structural Aspects of the Infrared Spectroscopy of Cellulose Acetate. **Tappi**, v. 45, n. 6, 1962.
- IOELOVICH, M. Characterization of Various Kinds of Nanocellulose. p. 51–100, 2017.
- ISO. **Pulps -Determination of Kappa number - ISO302:2004** International Organization Standardization. Geneva: International Organization for Standardization, 2004.
- JIN, E.; GUO, J.; YANG, F.; ZHU, Y.; SONG, J.; JIN, Y.; ROJAS, O. J. On the Polymorphic and Morphological Changes of Cellulose Nanocrystals (CNC-I) upon Mercerization and Conversion to CNC-II. **Carbohydrate Polymers**, v. 143, p. 327–335, 2016.
- JOHN, M. J.; THOMAS, S. **Biofibres and biocomposites** Carbohydrate Polymers, 2008.
- JONOBI, M.; OLADI, R.; DAVOUDPOUR, Y.; OKSMAN, K.; DUFRESNE, A.; HAMZEH, Y.; DAVOODI, R. Different Preparation Methods and Properties of Nanostructured Cellulose from Various Natural Resources and Residues: A Review. **Cellulose**, v. 22, n. 2, p. 935–969, Apr. 2015.
- KAFLE, K.; GREESON, K.; LEE, C.; KIM, S. H. Cellulose Polymorphs and Physical Properties of Cotton Fabrics Processed with Commercial Textile Mills for Mercerization and Liquid Ammonia Treatments. **Textile Research Journal**, v. 84, n. 16, p. 1692–1699, 1 Oct. 2014.
- KAMM, B.; KAMM, M. Biorefinery-Systems. **Chemical and biochemical engineering quarterly**, v. 18, n. 1, p. 1–6, 2004.
- KHANDELWAL, M.; WINDLE, A. H. Self-Assembly of Bacterial and Tunicate Cellulose Nanowhiskers. **Polymer**, v. 54, n. 19, p. 5199–5206, 2013.
- KHOSHKAVA, V.; KAMAL, M. R. Effect of Cellulose Nanocrystals (CNC) Particle Morphology on Dispersion and Rheological and Mechanical Properties of Polypropylene/CNC Nanocomposites. **ACS Applied Materials and Interfaces**, v. 6, n. 11, p. 8146–8157, 2014.
-

- KLEMM, D.; HEUBLEIN, B.; FINK, H. P.; BOHN, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. **Angewandte Chemie - International Edition**, v. 44, n. 22, p. 3358–3393, 2005.
- KLEMM, D.; KRAMER, F.; MORITZ, S.; LINDSTRÖM, T.; ANKERFORS, M.; GRAY, D.; DORRIS, A. **Nanocelluloses: A new family of nature-based materials** **Angewandte Chemie - International Edition**, 2011.
- KLEMM, D.; PHILIPP, B.; HEINZE, T.; HEINZE, U.; WAGENKNECHT, W. **Comprehensive Cellulose Chemistry**. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, 1998. v. 2.
- KÖHNKE, T.; LUND, K.; BRELID, H.; WESTMAN, G. Kraft Pulp Hornification: A Closer Look at the Preventive Effect Gained by Glucuronoxylan Adsorption. **Carbohydrate Polymers**, v. 81, n. 2, p. 226–233, 2010.
- KOLPAK, F. J.; BLACKWELL, J. Mercerization of Cellulose: 2. The Morphology of Mercerized Cotton Cellulose. **Polymer**, v. 19, n. 2, p. 132–135, 1978.
- KONTTURI, E. Hemicellulose: Structure, Characterization, Dissolution, Modification. n. November, 2015.
- KUMAR, A.; NEGI, Y. S.; CHOUDHARY, V.; BHARDWAJ, N. K. Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste. **Journal of Material Physics and chemistry**, v. 2, n. 1, p. 1–8, 2014.
- KUMAR, P.; KUMAR, P.; BARRETT, D. M.; BARRETT, D. M.; DELWICHE, M. J.; DELWICHE, M. J.; STROEVE, P.; STROEVE, P. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. **Industrial and Engineering Chemistry (Analytical Edition)**, v. 48, n. 8, p. 3713–3729, 2009.
- LANGAN, P.; NISHIYAMA, Y.; CHANZY, H. X-Ray Structure of Mercerized Cellulose II at 1 Å Resolution. **Biomacromolecules**, v. 2, n. 2, p. 410–416, 2001.
- LANGAN, P.; PETRIDIS, L.; O'NEILL, H. M.; PINGALI, S. V.; FOSTON, M.; NISHIYAMA, Y.; SCHULZ, R.; LINDNER, B.; HANSON, B. L.; HARTON, S.; HELLER, W. T.; URBAN, V.; EVANS, B. R.; GNANAKARAN, S.; RAGAUSKAS, A. J.; SMITH, J. C.; DAVISON, B. H. Common Processes Drive the Thermochemical Pretreatment of Lignocellulosic Biomass. **Green Chemistry**, v. 16, n. 1, p. 63, 2014.
- LARKIN, P. J. **“IR and Raman Spectroscopy - Principles and Spectral Interpretation.”**
- LAWRENCE, M.; JIANG, Y. Porosity, Pore Size Distribution, Micro-structure. In: AMZIANE, S.; COLLET, F. (Ed.). **Bio-aggregates based bu.** 1. Ed. RILEM, 2017. 23p. 39–71.
- LEAL, M. R. L. V.; WALTER, A. S.; SEABRA, J. E. A. Sugarcane as an Energy Source. **Biomass Conversion and Biorefinery**, v. 3, n. 1, p. 17–26, Mar. 2013.
-

- LENG, T. Cellulose Nanocrystals: Particle Size Distribution and Dispersion in Polymer Composites Tianyang Leng MSc Degree in Chemistry. 2016.
- LEPPÄNEN, K.; ANDERSSON, S.; TORKKELI, M.; KNAAPILA, M.; KOTELNIKOVA, N.; SERIMAA, R. Structure of Cellulose and Microcrystalline Cellulose from Various Wood Species, Cotton and Flax Studied by X-Ray Scattering. **Cellulose**, v. 16, n. 6, p. 999–1015, 2009.
- LIEBERT, T. Cellulose Solvents - Remarkable History, Bright Future. In: **Cellulose Solvents: For Analysis, Shaping and Chemical Modification**. 1033p. 3–54.
- LIGERO, P.; VEGA, A.; BAO, M. Acetosolv Delignification of Miscanthus Sinensis Bark: Influence of Process Variables. **Industrial Crops and Products**, v. 21, n. 2, p. 235–240, 2005.
- LIMA, P. R. L.; SANTOS, R. J.; FERREIRA, S. R.; TOLEDO FILHO, R. D. Characterization and Treatment of Sisal Fiber Residues for Cement-Based Composite Application. **Engenharia Agrícola**, v. 34, n. 5, p. 812–825, Oct. 2014.
- LOWELL, S.; SHIELDS, J. E.; THOMAS, M. A.; THOMMES, M. **Characterization of Porous Solids and Powders: Surface Area, Pore Size and Density**. Springer Netherlands, 2012.
- LU, P.; HSIEH, Y. Lo. Preparation and Characterization of Cellulose Nanocrystals from Rice Straw. **Carbohydrate Polymers**, v. 87, n. 1, p. 564–573, 2012.
- MACRELLI, S.; MOGENSEN, J.; ZACCHI, G. Techno-Economic Evaluation of 2nd Generation Bioethanol Production from Sugar Cane Bagasse and Leaves Integrated with the Sugar-Based Ethanol Process. **Biotechnology for Biofuels**, v. 5, n. 1, p. 22, 2012.
- MALONEY, T. C.; PAULAPURO, H. The Formation of Pores in the Cell Wall. **Journal of Pulp and Paper Science**, v. 25, n. 12, p. 430–436, 1999.
- MATHEW, A. P.; THIELEMANS, W.; DUFRESNE, A. Mechanical Properties of Nanocomposites from Sorbitol Plasticized Starch and Tunicin Whiskers. **Journal of Applied Polymer Science**, v. 109, n. 6, p. 4065–4074, 15 Sep. 2008.
- MEDRONHO, B.; LINDMAN, B. Competing Forces during Cellulose Dissolution: From Solvents to Mechanisms. **Current Opinion in Colloid & Interface Science**, v. 19, n. 1, p. 32–40, Feb. 2014.
- MENDES, F. M.; LING, L. Y.; DRIEMEIER, C. **Laboratory and Data Analysis Procedures for Determination of Biomass Nanoscale Porosity Employing Calorimetric Thermoporometry**. [s.l: s.n.].
- MOHAMAD HAAFIZ, M. K.; EICHHORN, S. J.; HASSAN, A.; JAWAID, M. Isolation and Characterization of Microcrystalline Cellulose from Oil Palm Biomass Residue. **Carbohydrate Polymers**, v. 93, n. 2, p. 628–634, 2013.
-

- MORAIS, J. P. S.; ROSA, M. D. F.; FILHO, M. D. S. M. de S.; NASCIMENTO, L. D.; NASCIMENTO, D. M. do; CASSALES, A. R. Extraction and Characterization of Nanocellulose Structures from Raw Cotton Linter. **Carbohydrate Polymers**, v. 91, n. 1, p. 229–235, 2013.
- MOSIER, N.; WYMAN, C.; DALE, B.; ELANDER, R.; LEE, Y. Y.; HOLTZAPPLE, M.; LADISCH, M. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. **Bioresource Technology**, v. 96, n. 6, p. 673–686, 2005.
- NASCIMENTO, D. M.; ALMEIDA, J. S.; DIAS, A. F.; FIGUEIRÊDO, M. C. B.; MORAIS, J. P. S.; FEITOSA, J. P. A.; ROSA, M. D. F. A Novel Green Approach for the Preparation of Cellulose Nanowhiskers from White Coir. **Carbohydrate Polymers**, v. 110, p. 456–463, Sep. 2014.
- NEGRO, M. J.; MANZANARES, P.; BALLESTEROS, I.; OLIVA, J. M.; CABAÑAS, A.; BALLESTEROS, M. Hydrothermal Pretreatment Conditions to Enhance Ethanol Production from Poplar Biomass. **Applied biochemistry and biotechnology**, v. 105–108, n. 1, p. 87–100, 2003.
- NISHIYAMA, Y.; SUGIYAMA, J.; CHANZY, H.; LANGAN, P. Crystal Structure and Hydrogen Bonding System in Cellulose I α from Synchrotron X-Ray and Neutron Fiber Diffraction. **J Am Chem Soc**, v. 125, p. 14300–14306, 2003.
- NISHIYAMA, Y.; KIM, U. J.; KIM, D. Y.; KATSUMATA, K. S.; MAY, R. P.; LANGAN, P. Periodic Disorder along Ramie Cellulose Microfibrils. **Biomacromolecules**, v. 4, n. 4, p. 1013–1017, 2003a.
- NISHIYAMA, Y.; LANGAN, P.; CHANZY, H. Crystal Structure and Hydrogen-Bonding System in Cellulose I β from Synchrotron X-Ray and Neutron Fiber Diffraction. **Journal of the American Chemical Society**, v. 124, n. 31, p. 9074–9082, Aug. 2002.
- NISHIYAMA, Y.; LANGAN, P.; O'NEILL, H.; PINGALI, S. V.; HARTON, S. Structural Coarsening of Aspen Wood by Hydrothermal Pretreatment Monitored by Small- and Wide-Angle Scattering of X-Rays and Neutrons on Oriented Specimens. **Cellulose**, v. 21, n. 2, p. 1015–1024, 2014.
- NISHIYAMA, Y.; SUGIYAMA, J.; CHANZY, H.; LANGAN, P. Crystal Structure and Hydrogen Bonding System in Cellulose I(Alpha) from Synchrotron X-Ray and Neutron Fiber Diffraction. **Journal of the American Chemical Society**, v. 125, n. 47, p. 14300–14306, 2003b.
- NOVO, L. P. **Determinação da relação dos parâmetros de solubilidade de Hansen de solventes orgânicos com a deslignificação organossolve de bagaço de cana-de-açúcar**. 2012. Universidade de São Paulo, 2012.
- NOVO, L. P.; GURGEL, L. V. A.; MARABEZEI, K.; CURVELO, A. A. da S. Delignification of Sugarcane Bagasse Using Glycerol-Water Mixtures to Produce Pulps for Saccharification. **Bioresource Technology**, v. 102, n. 21, p. 10040–10046, 2011.
-

- O'SULLIVAN, A. Cellulose: The Structure Slowly Unravels. **Cellulose**, v. 4, n. 3, p. 173–207, 1997.
- OKANOT, T.; SARK, A. Mercerization of Cellulose II Alkali-Cellulose Intermediates and a Possible Mercerization Mechanism. **Journal of Applied Polymer Science**, v. 30, p. 325–332, 1985.
- OKSMAN, K.; MATHEW, A. P.; BONDESON, D.; KVIEN, I. Manufacturing Process of Cellulose Whiskers/Polylactic Acid Nanocomposites. **Composites Science and Technology**, v. 66, n. 15, p. 2776–2784, 2006.
- OLIVEIRA, M. M. De. **Análise da porosidade nanométrica de materiais lignocelulósicos derivados de bagaço de cana-de-açúcar submetidos à compressão úmida**. 2014. Universidade de São Paulo, 2014.
- OLIVEIRA, R. P.; DRIEMEIER, C. CRAFS: A Model to Analyze Two-Dimensional X-Ray Diffraction Patterns of Plant Cellulose. **Journal of Applied Crystallography**, v. 46, n. 4, p. 1196–1210, 2013.
- PADMADISASTRA, Y.; GONDA, I. Preliminary Studies of the Development of a Direct Compression Cellulose Excipient from Bagasse. **Journal of pharmaceutical sciences**, v. 78, n. 6, p. 508–14, 1989.
- PARK, N.; KIM, H.; KOO, B.; YEO, H.; CHOI, I. Organosolv Pretreatment with Various Catalysts for Enhancing Enzymatic Hydrolysis of Pitch Pine (*Pinus Rigida*). **Bioresource Technology**, v. 101, n. 18, p. 7046–7053, Sep. 2010.
- PEREIRA, P. H. F.; ROSA, M. de F.; CIOFFI, M. O. H.; BENINI, K. C. C. de C.; MILANESE, A. C.; VOORWALD, H. J. C.; MULINARI, D. R.; PEREIRA, P. H. F.; ROSA, M. de F.; CIOFFI, M. O. H.; BENINI, K. C. C. de C.; MILANESE, A. C.; VOORWALD, H. J. C.; MULINARI, D. R. Vegetal Fibers in Polymeric Composites: A Review. **Polímeros**, v. 25, n. 1, p. 9–22, 2015.
- PU, Y.; ZHANG, J.; ELDER, T.; DENG, Y.; GATENHOLM, P.; RAGAUSKAS, A. J. Investigation into Nanocellulosics versus Acacia Reinforced Acrylic Films. **Composites Part B: Engineering**, v. 38, n. 3, p. 360–366, 2007.
- RÅNBY, B. G. The Colloidal Properties of Cellulose Micelles. **Discuss. Faraday Soc.**, v. 11, n. 111, p. 158–164, 1951.
- REN, J. L.; SUN, R. C. **Hemicelluloses**. 1. ed. Elsevier, 2010.
- RODRIGUEZ, N. L. G. de; THIELEMANS, W.; DUFRESNE, A. Sisal Cellulose Whiskers Reinforced Polyvinyl Acetate Nanocomposites. **Cellulose**, v. 13, n. 3, p. 261–270, 2006.
- ROJAS, O. J. **Cellulose Chemistry and Properties : Fibers , Nanocelluloses and Advanced Materials**.
-

- ROSA, M. D. F.; BEZERRA, F. C.; CORREIA, D.; SANTOS, F. J. de S.; ABREU, F. A. P.; FURTADO, A. A. L.; BRÍGIDO, A. K. L.; NORÕES, E. R. de V. **Utilização da Casca de Coco**. Fortaleza, Embrapa Agroindústria Tropical, , 2002.
- ROSA, M. F.; MEDEIROS, E. S.; MALMONGE, J. A.; GREGORSKI, K. S.; WOOD, D. F.; MATTOSO, L. H. C.; GLENN, G.; ORTS, W. J.; IMAM, S. H. Cellulose Nanowhiskers from Coconut Husk Fibers: Effect of Preparation Conditions on Their Thermal and Morphological Behavior. **Carbohydrate Polymers**, v. 81, n. 1, p. 83–92, 2010.
- ROWELL, R. M.; PETTERSEN, R.; TSHABALALA, M. A. Cell Wall Chemistry. In: **Handbook of Wood Chemistry and Wood Composites**.
- SADEGHIFAR, H.; FILPPONEN, I.; CLARKE, S. P.; BROUGHAM, D. F.; ARGYROPOULOS, D. S. Production of Cellulose Nanocrystals Using Hydrobromic Acid and Click Reactions on Their Surface. **Journal of Materials Science**, v. 46, n. 22, p. 7344–7355, 2011.
- SANNIGRAHI, P.; MILLER, S. J.; RAGAUŠKAS, A. J. Effects of Organosolv Pretreatment and Enzymatic Hydrolysis on Cellulose Structure and Crystallinity in Loblolly Pine. **Carbohydrate research**, v. 345, n. 7, p. 965–70, 2010.
- SCHÄDEL, C.; BLÖCHL, A.; RICHTER, A.; HOCH, G. Quantification and Monosaccharide Composition of Hemicelluloses from Different Plant Functional Types. **Plant Physiology and Biochemistry**, v. 48, n. 1, p. 1–8, 2010.
- SHELLER, H. V.; ULVSKOV, P. Hemicelluloses. **Annual Review of Plant Biology**, v. 61, n. 1, p. 263–289, 2010.
- SCZOSTAK, A. Cotton Linters: An Alternative Cellulosic Raw Material. **Macromolecular Symposia**, v. 280, n. 1, p. 45–53, 2009.
- SÈBE, G.; HAM-PICHAVENT, F.; IBARBOURE, E.; KOFFI, A. L. C.; TINGAUT, P. Supramolecular Structure Characterization of Cellulose II Nanowhiskers Produced by Acid Hydrolysis of Cellulose i Substrates. **Biomacromolecules**, v. 13, n. 2, p. 570–578, 2012.
- SIAUEIRA, G.; BRAS, J.; DUFRESNE, A. Cellulose Whiskers versus Microfibrils: Influence of the Nature of the Nanoparticle and Its Surface Functionalization on the Thermal and Mechanical Properties of Nanocomposites. **Biomacromolecules**, v. 10, n. 2, p. 425–432, 2009.
- SIQUEIRA, G.; DUFRESNE, A. Ramie and Luffa Cylindrica Nanowhiskers as Reinforced Phase in Polycaprolactone. **Print Media**, p. 1–10, 2010.
- SIXTA, H. Part I-Chemical Pulping. In: SIXTA, H. (Ed.). **Handbook of Pulp**. 1. ed. [s.l.] Wiley-VCH Verlag GmbH & Co. KGaA, 2006. p. 1–20.
- SOUZA LIMA, M. M. De; BORSALI, R. **Rodlike cellulose microcrystals: Structure, properties, and applications** **Macromolecular Rapid Communications**, 2004.
- SPIRIDON, I.; POPA, V. I. Hemicelluloses: Structure and Properties.
-

- STEWART, D.; AZZINI, A.; HALL, A. T.; MORRISON, I. M. Sisal Fibres and Their Constituent Non-Cellulosic Polymers. **Industrial Crops and Products**, v. 6, n. 1, p. 17–26, 1997.
- SUN, R.; HUGHES, S. Fractional Extraction and Physico-Chemical Characterization of Hemicelluloses and Cellulose from Sugar Beet Pulp. **Carbohydrate Polymers**, v. 36, n. 4, p. 293–299, 1998.
- SWATLOSKI, R. P.; SPEAR, S. K.; HOLBREY, J. D.; ROGERS, R. D. Dissolution of Cellulose with Ionic Liquids. **Journal of the American Chemical Society**, v. 124, n. 18, p. 4974–4975, 2002.
- SZCZERBOWSKI, D.; PITARELO, A. P.; ZANDONÁ FILHO, A.; RAMOS, L. P. Sugarcane Biomass for Biorefineries: Comparative Composition of Carbohydrate and Non-Carbohydrate Components of Bagasse and Straw. **Carbohydrate Polymers**, v. 114, p. 95–101, 2014.
- TAHERZADEH, M. J.; KARIMI, K. **Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review** *International Journal of Molecular Sciences*, 2008.
- TAN, I. A. W.; AHMAD, A. L.; HAMEED, B. H. Optimization of Preparation Conditions for Activated Carbons from Coconut Husk Using Response Surface Methodology. **Chemical Engineering Journal**, v. 137, n. 3, p. 462–470, 2008.
- TAPPI. Micro Kappa Number UM 246. **Tappi**, p. 2, 1991.
- TAPPI. Kappa Number of Pulp T236 Om-99. **Tappi**, p. 1–4, 1999.
- TAPPI. **Ash in wood , pulp , paper and paperboard : combustion at 525 ° C-T211 om-02**.
- TAPPI. **Workshop on International Standards for Nanocellulose**, Arlington, 2011a.
- TAPPI. Acid-Insoluble Lignin in Wood and Pulp. **Test**, p. 3–7, 2011b.
- TEIXEIRA, E. de M.; BONDANCIA, T. J.; TEODORO, K. B. R.; CORRÊA, A. C.; MARCONCINI, J. M.; MATTOSO, L. H. C. Sugarcane Bagasse Whiskers: Extraction and Characterizations. **Industrial Crops and Products**, v. 33, n. 1, p. 63–66, Jan. 2011.
- TRAN, H.; VAKKILAINEN, E. K. The Kraft Chemical Recovery Process. **TAPPI Kraft Recovery Course**, p. 1–8, 2012.
- VAN DAM, J. E. G.; VAN DEN OEVER, M. J. A.; TEUNISSEN, W.; KEIJERS, E. R. P.; PERALTA, A. G. Process for Production of High Density/High Performance Binderless Boards from Whole Coconut Husk. Part 1: Lignin as Intrinsic Thermosetting Binder Resin. **Industrial Crops and Products**, v. 19, n. 3, p. 207–216, 2004.
- VAN DILLEWIJN, C. **Botany of sugarcane**. Chronica Botanica Co., 1952.
- VARSHNEY, V. K.; NAITHANI, S. **Cellulose Fibers: Bio- and Nano-Polymer Composites**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011.
-

- VÁZQUEZ, G.; ANTORRENA, G.; GONZÁLEZ, J.; FREIRE, S.; LÓPEZ, S. Acetosolv Pulping of Pine Wood. Kinetic Modelling of Lignin Solubilization and Condensation. **Bioresource Technology**, v. 59, n. 2–3, p. 121–127, 1997.
- WADA, M.; CHANZY, H.; NISHIYAMA, Y.; LANGAN, P. Cellulose III I Crystal Structure and Hydrogen Bonding by Synchrotron X-Ray and Neutron Fiber Diffraction. **Macromolecules**, v. 37, n. 23, p. 8548–8555, 2004.
- WADA, M.; HEUX, L.; SUGIYAMA, J. Polymorphism of Cellulose I Family: Reinvestigation of Cellulose IVI. **Biomacromolecules**, v. 5, n. 4, p. 1385–1391, 2004.
- WANG, N.; DING, E.; CHENG, R. Thermal Degradation Behaviors of Spherical Cellulose Nanocrystals with Sulfate Groups. **Polymer**, v. 48, n. 12, p. 3486–3493, 2007.
- WANG, W.; CHEN, X.; DONOHOE, B. S.; CIESIELKI, P. N.; KATAHIRA, R.; KUHN, E.; KAFLE, K.; LEE, C. M.; PARK, S.; KIM, S. H.; TUCKER, M. P.; HIMMEL, M. E.; JOHNSON, D. Effect of Mechanical Disruption on the Effectiveness of Three Reactors Used for Dilute Acid Pretreatment of Corn Stover Part 1: Chemical and Physical Substrate Analysis. **Biotechnology for Biofuels**, v. 57, n. 7, p. 47, 2014.
- WEISE, U.; MALONEY, T.; PAULAPURO, H. **Quantification of water in different states of interaction with wood pulp fibres Cellulose**, 1996.
- Y. LIN, S. Accessibility of cellulose: a critical review. **Fibre Science and Technology**, 1972.
- ZAINI, L. H.; TAHIR, P. M.; KARIMI, S. Isolation and Characterization of Cellulose Whiskers from Kenaf (*Hibiscus Cannabinus* L.) Bast Fibers. **Journal of Biomaterials and Nanobiotechnology**, v. 04, n. January, p. 37–44, 2013.
- ZHAO, X.; QI, F.; LIU, D. Hierarchy Nano- and Ultrastructure of Lignocellulose and Its Impact on the Bioconversion of Cellulose. In: **Nanotechnology for Bioenergy and Biofuel Production**.
-